Environmental Health Perspectives
Vol. 82, pp. 125–163, 1989

Multiple-Site Carcinogenicity of Benzene in Fischer 344 Rats and B6C3F$_1$ Mice

by J. E. Huff,* Joseph K. Haseman,* David M. DeMarini,† Scot Eustis,* Robert R. Maronpot,* Arthur C. Peters,‡ Ronald L. Persing,‡ Clarence E. Chrisp,§ and Abigail C. Jacobs**

Toxicology and carcinogenesis studies of benzene (CAS No. 71-43-2; greater than 99.7% pure) were conducted in groups of 60 F344/N rats and 60 B6C3F$_1$ mice of each sex for each of three exposure doses and vehicle controls. These composite studies on benzene were designed and conducted because of large production volume and widespread human exposure, because of the epidemiologic association with leukemia, and because previous experiments were considered inadequate or inconclusive for determining carcinogenicity in laboratory animals. Using the results from 17-week studies, doses for the 2-year studies were selected based on clinical observations (tremors in higher dosed mice), on clinical pathologic findings (lymphoid depletion in rats and leukopenia in mice), and on body weight effects. Doses of 0, 50, 100, or 200 mg/kg body weight benzene in corn oil were administered by gavage to male rats, 5 days per week, for 103 weeks. Doses of 0, 25, 50, or 100 mg/kg benzene in corn oil were administered by gavage to female rats and to male and female mice for 103 weeks. Ten animals in each of the 16 groups were killed at 12 months, and necropsies were performed. Hematologic profiles were performed at 3-month intervals.

For the 2-year studies, mean body weights of the top dose groups of male rats and of both sexes of mice were lower than those of the controls. Survivals of the top dose group of rats and mice of each sex were reduced; however, at week 92 for rats and week 91 for mice, survival was greater than 60% in all groups; most of the dosed animals that died before week 103 had neoplasia. Compound-related nonneoplastic or neoplastic effects on the hematopoietic system, Zymbal gland, forestomach, and adrenal gland were found both for rats and mice. Further, the oral cavity was affected in rats, and the lung, liver, Harderian gland, preputial gland, ovary, and mammary gland were affected in mice. Under the conditions of these 2-year gavage studies, there was clear evidence of carcinogenicity of benzene in male F344/N rats, female F344/N rats, male B6C3F$_1$ mice, and female B6C3F$_1$ mice. In male rats, benzene caused increased incidences of Zymbal gland carcinomas, squamous cell papillomas and squamous cell carcinomas of the oral cavity, and squamous cell papillomas and squamous cell carcinomas of the skin. In female rats, benzene caused increased incidences of Zymbal gland carcinomas and squamous cell papillomas and squamous cell carcinomas of the oral cavity. In male mice, benzene caused increased incidences of Zymbal gland squamous cell carcinomas, malignant lymphomas, alveolar/bronchiolar carcinomas and alveolar/bronchiolar adenomas or carcinomas (combined), Harderian gland adenomas, and squamous cell carcinomas of the preputial gland. In female mice, benzene caused increased incidences of malignant lymphomas, ovarian granulosa cell tumors, ovarian benign mixed tumors, carcinomas and carcinosarcomas of the mammary gland, alveolar/bronchiolar adenomas, alveolar/bronchiolar carcinomas, and Zymbal gland squamous cell carcinomas. Dose-related lymphocytopenia was observed for male and female F344/N rats and male and female B6C3F$_1$ mice. These unequivocal observations show clearly that benzene is a trans-species, trans-sex, multisite potent carcinogen.

*National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, NC 27709.
†U.S. Environmental Protection Agency, Research Triangle Park, NC 27711.
‡Battelle Columbus Division, Columbus, OH 43201.
§University of Michigan, Ann Arbor, MI 48109.
**Carltech Associates, Inc., Rockville, MD 20852.
Address reprint requests to J. E. Huff, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, NC 27709.

Introduction

This paper presents the key nonneoplastic and neoplastic findings from our long-term benzene exposure studies in rats and mice (*1*,*2*), as first announced and peer reviewed in 1983 at a public meeting held at the National Institute of Environmental Health Sciences. Also given are selected details about the experimental design, study conduct, histopathologic diagnoses, statistical analyses,

data interpretations, and conclusions. Relevant background information regarding benzene has been included on production and use, exposure and standards, human health effects, genetic toxicology, and carcinogenesis studies of benzene metabolites. Benzene represents one of nearly 400 chemical-specific, long-term carcinogenesis studies conducted in rodents and reported in a series of technical reports by the National Cancer Institute, for the first 200 chemicals (*3,4*), and by the National Toxicology Program for the subsequent 175 chemicals (*5,6*).

Production, Use, and Occurrence

Benzene ranks 16th in production volume for chemicals produced in the United States, with approximately 11.84 billion pounds being produced in 1988, 11.7 billion pounds in 1987, 10.2 billion pounds in 1986, and 9.4 billion pounds in 1985 (*7*). Considering only organic chemicals benzene places sixth. This simplest aromatic chemical is used primarily as a raw material in the synthesis of styrene (polystyrene plastics and synthetic rubber), phenol (phenolic resins), cyclohexane (nylon), aniline, maleic anhydride (polyester resins), alkylbenzenes (detergents), chlorobenzenes, and other products used in the production of drugs, dyes, insecticides, and plastics (*8*). Benzene, along with other light, high-octane aromatic hydrocarbons, such as toluene and xylenes, is a component of motor gasoline. Benzene is also used as a solvent, but for most applications, it has been replaced by what are considered less hazardous solvents.

Benzene has a long history of extensive use in industry, first as a volatile solvent and later as a starting material for the synthesis of other chemicals. An aromatic fraction containing benzene was known in the 18th century as a product of the distillation of coal (*8*). Benzene, or bicarburet of hydrogen as it was then called, was first isolated in 1825 by Faraday, who obtained it from a liquid condensed by compressing oil gas. In 1833, Mitscherlich obtained bicarburet of hydrogen by distilling benzoic acid with lime and suggested the name "benzin" for the compound. Leibig objected and proposed "benzole." In 1845, benzene was found by Hoffman in light oil derived from coal tar. The commercial recovery of benzene from this source was developed and described by Mansfield in 1849. The synthesis of benzene by the polymerization of acetylene was first carried out by Berthelot in 1866. Discovery of benzene in coal gas after 1876 initiated the recovery of coal gas light oil as a source of benzene. Although petroleum was known to contain benzene, recovery of this material was not undertaken on a commercial scale until about 1941. Several years after the end of World War II, the demand for benzene by the rapidly expanding chemical industry exceeded the total production by the coal carbonization industry. To help meet this deficit, benzene was produced in ever increasing amounts by the petroleum and petrochemical industries by the recovery from reformate and liquid byproducts and ethylene manufacture. Today, production from these sources far exceeds that from coal.

Benzene, the parent hydrocarbon of a series of aromatic compounds, should not be confused with the product benzin or benzine, a comparatively low boiling point petroleum fraction consisting of a mixture of hydrocarbons, predominantly aliphatic in type. The term "benzol" is used to describe commercial products that are largely benzene; the term is rarely seen in the United States but is still found in British publications. The pure compound is now called benzene, the name approved by the International Union of Pure and Applied Chemistry and adopted by industries and nomenclature experts.

Benzene recovered from both petroleum and coal sources is extracted from catalytic reformate made in oil refineries, from pyrolysis gasoline made in steam-cracking olefin plants, from light oils in coking coal, and from dealkylation of toluene (*9*). The major derivatives of benzene are ethylbenzene (50%), cumene (15%), cyclohexane (15%), and aniline (5%). Primary end uses include polystyrene (25%), nylon (20%), other styrenic polymers (10%), and rubber (5%). As an antiknock chemical, benzene and benzene-enriched aromatics are added to gasoline as a replacement for alkyl lead compounds. The benzene content of European motor fuel is about 5% (*10*); U.S. gasoline contains an average of 0.8% (*11*).

Benzene is apparently ubiquitous in the environment. The main source of benzene in the environment is from industrial processes. However, benzene is a natural constituent of crude oil (*12*). Early uses of benzene as a solvent regularly resulted in workplace air concentrations of 1600 to 3200 mg/m^3 (approximately 500 to 1000 ppm) and above (*11*). In addition to the major source of benzene in the environment (industry), benzene is found in air, water, and sediments; soil and plants; food, beverages, and feed; tobacco and tobacco smoke; and pyrolysis products.

Human Exposure

Hunter chronicles the history of long-term benzene exposure (*13*); a few early facts are given here for historic interest. In the last years of the 19th century, the use of benzene as a rubber solvent led to small outbreaks of "purpura hemorrhagica" with a number of deaths from aplastic anemia. Selling was the first to associate benzene poisoning with leukopenia (*14*). Unfortunately, this association led to using benzene in the treatment of leukemia; benzene was given in gelatin capsules starting with 3 g per day and increasing to 5 g per day. The result was a gain in weight, shrinkage of the spleen, and great reduction of the white cell count. After some months of treatment, however, most patients developed multiple hemorrhages, especially from the fauces and gums; women experienced troublesome menorrhagia. With advancing anemia (loss of white and red blood cells), fever, and bleeding, these patients died of chronic poisoning. Fortunately, this clinical application was discontinued by 1913. In most industrial countries, the use of benzene as a solvent increased after World War I. In 1922, Alice Hamilton began her efforts to reduce the hazards of benzene in American industry. Early recognition of the seri-

ous adverse health effects of benzene (*1*) stimulated many industries in 1928 to change their attitudes and use safer alternatives for benzene, even when more expensive (*13*). Toluene was substituted for benzene in dry cleaning and in thinners used for quick-drying paints. Cellulose lacquers sprayed onto motor-car bodies were dissolved in toluene instead of benzene. Current toxicological data support the substitution decisions made in the 1920s. However, in 1939 three plants were found that used about 50,000 gallons of benzene per month, and benzene concentrations in the workrooms ranged from 11 to 1060 ppm (*13*). Of the 332 workers examined, 130 showed varying degrees of benzene poisoning. The use of benzene was discontinued in these three rotogravure operations, and other solvents were used.

Workplace and environmental exposure to benzene is widespread. As many as 2 million workers may be exposed (*12,15*). Nonoccupational exposure to benzene results primarily from environmental contamination, the chief sources being automobile refueling operations and emissions, consumer products, industrial emission, and cigarette smoke. Benzene intake by urban residents in the U.S. is approximately 850 μg/day (*16*). For example, benzene is present in cigarette smoke at concentrations of 47 to 64 ppm (*17*); dietary intake may be as high as 250 μg/day (*16*). Benzene has also been identified in bottled artesian water (*18*) and in boiled beef and canned beef stew (*19*). The average background benzene concentration in urban areas ranges from about 1.5 to 6 ppb (*20*).

Two studies have shown considerable benzene absorption via skin. Susten et al. (*21*), using hairless mice, calculated that 20 to 40% of the total benzene dose received by humans in tire-building operations could be absorbed dermally. Blank and McAuliffe (*22*) calculated that an adult working in ambient air containing 10 ppm benzene would absorb 7.5 μL/hr from inhalation and 1.5 μL/hr from whole body (2 m^2) exposure. They also calculated that 100 cm^2 glabrous skin in contact with gasoline containing 5% benzene would absorb 7.0 μL/hr. These results are not too different from those of Susten et al. using mouse skin (*21*).

Exposure Standard

Adopted in 1971, the Occupational Safety and Health Administration (OSHA) standard for benzene was an 8-hr time-weighted average (TWA) of 10 ppm with a ceiling limit of 25 ppm and a maximum peak concentration of 50 ppm for a 10-min period (*23*). The standard apparently was based on concern about the development of aplastic anemia and depression of various cellular elements in the blood, but not on concern about the development of cancer. In May 1977, OSHA issued an Emergency Temporary Standard that reduced exposures to benzene to 1 ppm and included other provisions. In February 1978, OSHA promulgated a new permanent standard for occupational exposure to benzene based on evidence that there was a causal connection between benzene exposure and leukemia. This standard limited employee exposure to 1 ppm as an 8-hr TWA, then estimated to be the lowest feasible level. The standard included a ceiling limit of 5 ppm for any 15-min period during an 8-hr day, limits on eye and skin contact with benzene, and industrial hygiene and medical surveillance provisions.

The United States Court of Appeals for the Fifth Circuit vacated the standard in 1978, and the Supreme Court affirmed that judgment in 1980. The Supreme Court held that OSHA's prior approach (reducing exposures to the lowest feasible level when there was strong qualitative evidence of carcinogenicity) was insufficient. In December 1985, OSHA issued a newly proposed rule and notice of hearing on occupational exposure to benzene (*23*). In this announcement, OSHA "proposes to reduce the existing benzene permissible exposure limit of 10 parts benzene per million parts of air (10 ppm) to an eight (8)-hour time-weighted average of 1 ppm to reduce substantially a significant health risk." The then current 10-ppm exposure limit in the U.S. corresponded to that in most other industrialized countries, except for Sweden (5 ppm) and the USSR (5 mg/m^3, approximately equivalent to 2 ppm) (*24*).

In September 1987, OSHA announced a final rule on occupational exposure to benzene: "The revised standard reduces the permissible exposure limit (PEL) from 10 parts benzene per million parts of air (10 ppm) to an eight (8)-hour time-weighted average (TWA) of 1 ppm and a short-term exposure limit (STEL) of 5 ppm [reduced from 25 ppm]. An action level of 0.5 ppm is established to encourage lower exposures for employees and to reduce administrative burdens to employers. This standard applies to all industries covered by the Occupational Safety and Health Act" (*25*).

Metabolism and Excretion

After monosubstitution of benzene has occurred, three disubstitution positions are chemically possible: 1,2- (*ortho*); 1,3- (*meta*); or 1,4- (*para*). Electron-withdrawing groups promote *meta* substitutions and electron donating groups favor *ortho* and *para* substitutions. Since most benzene metabolites are hydroxyl additions, the *ortho para* positions predominate (*8*). Following metabolic formation of phenol (C_6H_5OH), further metabolism leads to both catechol (pyrocatechol; *ortho*-dihydroxybenzene) and hydroquinone (*para*-dihydroxybenzene). The third possible form, *meta*-dihydroxybenzene (resorcinol), probably does not occur. As described by Irons and Pfeifer (*26*), the primary oxidation of benzene occurs via the cytochrome P-450-dependent monooxygenase system, resulting in biologically reactive intermediates. As generally agreed, benzene *per se* does not represent the principal structural moiety causing the identified toxic effects on the bone marrow or lymphoid system (*26,27*). The metabolism and elimination of benzene in humans appear to be similar to those in rats and mice; the amounts of various metabolites, the extent of metabolism, and the nature of the phenol conjugates depend on the species, strain, and the route of administration (*28*).

Comparative metabolism and disposition studies of benzene and its metabolites in selected tissues in F344 rats and B6C3F$_1$ mice are being conducted by the oral and inhalation routes (*29–32*).

Genetic Toxicology of Benzene

The genetic toxicology of benzene and other solvents (toluene, xylenes, phenols) has been summarized by Dean (*33*) and Kalf (*34*). This section highlights the past and current literature.

Bacterial Systems

Many studies have shown that benzene is not mutagenic in bacteria. Eight strains of Salmonella have been used, and rat liver S9 has been prepared from uninduced animals and from animals induced with 3-methylcholanthrene, phenobarbital, or Aroclor 1254 (*35–43*). Although all of these studies used the standard plate-incorporation assay described by Ames et al. (*44*), negative results were obtained in a host-mediated assay that consisted of strain TA1950 injected IP into Swiss albino mice; the mice then received two SC 0.1-mL injections of benzene at 1-hr intervals (*45*). Bartsch et al. (*46*) exposed strains TA98 and TA100 in inverted Petri dishes at concentrations less than or equal to 20% benzene (v/v) in air in a 10-L desiccator for 4 or 12 hr at 37°C. The results were negative in the presence or absence of Aroclor-induced rat liver S9. Similarly, Bos et al. (*47*) used the taped-plate assay for volatile compounds and obtained negative results.

Although all of these studies were reverse-mutation assays at a histidine gene in Salmonella, a forward-mutation assay in Salmonella for resistance to 8-azaguanine also failed to detect any mutagenic activity of benzene (*48,49*). Benzene not only failed to revert a variety of strains of Salmonella but also did not revert a histidine auxotroph of *Bacillus subtilis* (*50*). Although McCarroll (*51,52*) reported that benzene caused growth inhibition by producing DNA damage in *B. subtilis* and *Escherichia coli*, Rosenkranz and Leifer (*53*) found that benzene was negative in the *E. coli* pol A test. Likewise, benzene did not cause DNA damage in SOS-induction assays in *E. coli* (*43*) or *S. typhimurium* (*54*).

Nonmammalian Eukaryotic Systems

Benzene failed to induce petites and gene conversion/crossing over in yeast (*55,56*); however, it did induce aneuploidy in yeast (*57*). Benzene was negative for somatic mutation in *Drosophila melanogaster* in an early study by Nylander et al. (*58*); however, it was positive in the wing-spot assay (*59*). Benzene was generally negative for germ cell mutation (sex-linked recessive lethals) in Drosophila (*60,61*), and the results have been summarized by Vogel (*62*). Exposure of the vascular plant Tradescantia resulted in mutations (*63,64*), and benzene caused chromosomal anomalies in the grasshopper (*65*).

Mammalian Cells *In Vitro*

Benzene was not mutagenic in the mouse lymphoma forward-mutation assay in L5178Y/TK$^{+/-}$ cells (*66*). In a series of studies summarized by Garner (*67*), benzene was not mutagenic in a variety of cell lines from mouse (L5178Y), hamster (CHO, V79), and human (TK6) at various loci (*hprt, tk, oua*^{r}). Three studies in primary rat hepatocytes (*68–70*) and two in HeLa cells (*71,72*) found no evidence that benzene could induce unscheduled DNA synthesis (UDS), which is an indicator of DNA damage. However, Glauert et al. did find evidence that benzene induced UDS in primary rat hepatocytes (*73*). Benzene also induced DNA strand breakage in L5178Y mouse lymphoma cells (*74*).

Koizumi et al. (*75*) found that benzene induced chromosome breaks and gaps in cultured human leukocytes, but high doses and long exposure times were required to produce the chromosomal abnormalities. Morimoto reported that treatment of cultured human lymphocytes with benzene at high doses for long exposure times caused chromosomal abnormalities (*76*), including acentric fragments, breaks, and gaps. Howard et al. also found that benzene induced chromosomal aberrations in human lymphocytes (*77*). These observations were not confirmed by Gerner-Smidt and Friedrich (*78*), who treated cultured human lymphocytes with benzene for 72 hr and found no increase in chromosomal aberrations. Two studies found that benzene induced chromosomal aberrations in hamster cells (*79,80*), but these were not confirmed in other studies nor in other cell types (*81*).

Several studies found that benzene did not induce SCEs in human lymphocytes (*78,80,82*) or rodent cells (*81*). However, with increased levels of S9 or benzene, three studies found that benzene induced SCEs in human lymphocytes (*83–85*). Benzene also was found to induce aneuploidy in Chinese hamster liver cells (*86*).

Benzene induced mammalian cell transformation in SHE cells (*87–89*). However, it was negative in a number of other cell transformation assays (*90*).

Mammals *In Vivo*

Although benzene has given mixed results for the induction of SCEs and for chromosomal aberrations *in vitro*, it is one of the few compounds that has been tested extensively for a variety of cytogenetic effects *in vivo*. There are now many studies that show that benzene causes cytogenetic damage in rodents *in vivo*; of these, 13 are in mice, 5 are in rats, and 1 is in rabbits. However, benzene failed to induce cytogenetic damage in hamsters.

Micronuclei. Micronuclei are chromosomes or small fragments of chromosomes that are not incorporated into daughter nuclei during cell division. They may be induced by agents that break chromosomes (clastogens) or that affect the spindle apparatus. The ability of benzene to induce micronuclei in the bone marrow polychromatic erythrocytes of mice has been confirmed by numerous independent studies in various strains of mice.

Diaz et al. (*91*) and Tunek et al. (*92*) administered ben-

zene SC to male mice (the F_1 from the cross CSW × Cs No. 1 or outbred NMRI) and observed a dose-dependent increase in micronuclei. Benzene was administered by gavage to male and female mice in the following three studies. Hite et al. (*93*) found that benzene induced similar dose-dependent increases in micronuclei in both sexes of Charles River (CD-1) mice. Meyne and Legator (*94*) and Siou et al. (*95*), who used Swiss (CD-1) and Swiss Lane Petter mice, respectively, also found that benzene induced micronuclei in both sexes, but with greater dose-dependent increases in micronuclei in males than in females. This was confirmed by Gad-El-Karim et al. (*96*). Siou et al. (*95*) showed that castration of male mice reduced their sensitivity for micronuclei formation below that of the females. Meyne and Legator (*94*) found that IP injections of benzene produced a dose-dependent increase in micronuclei in male mice and yet caused no increase in micronuclei in females. The NTP found that benzene administered by gavage induced micronuclei in male and female $B6C3F_1$ mice; males were more sensitive than females (*97*). Additional evidence of the ability of benzene to induce micronuclei in mice has been found by Gad-El-Karim et al. (*98*) and Harper and Legator (*99*). An inhalation study in male DBA/2 mice found that benzene induced micronuclei in bone marrow after exposure to a single target concentration of just 10 ppm of benzene for 6 hr (*100*).

In male Long-Evans rats, IP injections of benzene induced a dose-dependent increase in micronuclei, and the doses were similar to those that induced micronuclei in mice (*45*). Micronuclei were also induced in bone marrow of male Sprague-Dawley rats (11–14 weeks old) exposed by inhalation to a target concentration of benzene as low as 1 ppm for 6 hr (*100*). Although benzene induced micronuclei in mice and rats, Siou et al. (*95*) did not find an increase in micronuclei in hamsters that received benzene by gavage.

Chromosomal Aberrations. Meyne and Legator (*94*) reported that exposure of Swiss (CD-1) mice to benzene by gavage or IP injection increased the frequency of chromosomal aberrations and that males were more sensitive than females. However, Tice et al. (*101*) found that exposure of DBA/2 mice to benzene by inhalation caused an increase in chromosomal aberrations only when the animals had been pretreated with phenobarbital. The authors also showed that the combination of benzene and phenobarbital inhibited cellular proliferation in the bone marrow; once again, males were more sensitive to this inhibition than were females. Subsequent studies (*102*) showed that the combination of benzene and phenobarbital enhanced the frequency of chromosomal aberrations in males more than in females. The aberrations were all of the chromatid type, not of the chromosome type, and there were no increases in chromosomal rearrangement of any kind. Siou et al. (*95*) confirmed the observations of Meyne and Legator (*94*) by administering benzene by gavage to Swiss Lane Petter mice: Chromosome gaps were enhanced in both sexes, and males were more sensitive than females. Gad-El-Karim et al. (*96*) found that benzene induced chromosomal aberrations in CD-1 mice, and Rithidech et al. (*103*) found that benzene induced chromosomal aberrations in lymphocytes of Swiss (ICR) male mice.

Four reports show that benzene induces chromosomal aberrations in rats. Lyon (*45*) injected benzene IP into male Long-Evans rats and found a significant increase in chromosomal aberrations in bone marrow cells, including achromatic lesions, chromatid and chromosome deletions, and double minutes (small supernumerary chromosomal fragments). Dean (*104*) reported that benzene injected IP to Carworth CF_1 rats increased the number of chromatid gaps and chromosome fragments in bone marrow cells from both sexes. Anderson and Richardson (*105*) examined the ability of benzene to induce chromosomal aberrations in the bone marrow cells of Wistar-derived male Alderly Park rats. Exposure by inhalation or injection increased the frequency of chromosomal aberrations. No dose response was observed, and a single exposure was as effective as multiple doses. Styles and Richardson (*106*) found that benzene induced chromosomal aberrations in male Wistar-derived rats when exposed by inhalation to benzene at 100 ppm for 6 hr.

A significant increase in chromosomal aberrations, mostly gaps and breaks, was found in rabbits injected SC with benzene (*107*). Although benzene induces chromosomal aberrations in mice, rats, and rabbits, a similar effect has not been seen in Chinese hamsters. Siou et al. (*95*) administered benzene by gavage to Chinese hamsters and reported no increase in chromosomal aberrations.

Sister Chromatid Exchanges. The ability of benzene to induce SCEs in mice was reported by Tice et al. (*101,102*). Inhaled benzene enhanced the frequency of SCEs in male and female DBA/2 mice, but more so in males. Three-month-old mice were more sensitive than were 10-month-old mice. The authors also compared effects resulting from two different routes of exposure and found that inhalation of 120 ppm of benzene for 4 hr equaled the effect of an IP injection of 1 mmole/kg. The authors have tentatively concluded that a greater increase in SCEs resulted from inhaled benzene than from injected benzene. In contrast to their other findings, these authors also found that treatment with phenobarbital before inhalation of benzene enhanced SCEs in male and female mice but more so in females. Inhaled benzene also caused a greater increase in SCEs in DBA/2 mice than in C57BL/6 mice. These two strains are isogenic and vary in that DBA/2 mice have no inducible aryl hydrocarbon hydroxylase (AHH) activity, whereas the C57BL/6 strain does. Erexson et al. (*100*) found that inhalation of 10 ppm by male DBA/2 mice or 3 ppm by male Sprague-Dawley rats resulted in significant increases in SCE frequencies in peripheral blood lymphocytes.

Other End Points. An additional end point, sperm-head abnormalities, has been studied by Topham (*108*), who showed that hybrid mice (CBA × BALB/c) given IP injections of benzene exhibited small but reproducible and significant increases in sperm-head abnormalities. Earlier, Lyon (*45*) found that IP injections of benzene in Long-Evans rats at 0.5 mL/kg (one-fifth the LD_{50} value) did not increase the frequency of dominant lethals.

Cytogenetic Studies in Humans

Benzene is one of the few agents whose cytogenetic effects have been studied in humans. The populations studied can be divided into two general groups: people with a current or past history of benzene-associated blood dyscrasias, and workers with current or past exposure to benzene but with no apparent clinical signs of blood dyscrasias. In many of the 23 studies (*11*), significant increases in chromosomal aberrations were observed, which in some cases persisted for years after exposure ceased. Most of these studies involved small numbers of humans exposed to benzene and did not present detailed information about the concentration and length of exposure. Although the data do not support a clear association between exposure to benzene and persistent chromosomal aberrations, the data do indicate an association between benzene-associated hemopathies and chromosomal aberrations (*33,34*).

In summary, these studies show that benzene is clearly a clastogen and that benzene causes other chromosomal changes. It is not clear if benzene is a gene mutagen, an agent that causes a molecular change that affects only a single gene, as opposed to a chromosomal (multiple gene) mutagen. In addition, benzene must be metabolized *in vivo* to cause chromosomal changes, suggesting that metabolites of benzene are responsible for any observed cytogenetic changes. In general, male rodents are more sensitive than females to the genotoxic effects of benzene, and the route of administration influences the ability of benzene to induce chromosomal damage. Cytogenetic damage also has been observed in humans who have developed benzene-associated hemopathies, especially leukemia.

Fetoxicity and Teratogenicity

Cleft palate, agnathia, and micrognathia were associated with a single SC injection of 3 mL/kg to CF-1 mice on day 13 of gestation (*109*). This study was considered to be inadequate, however, because no controls were used. Delayed ossification of sternebrae was observed in the offspring of Sprague-Dawley rats exposed to air containing 300 or 2200 ppm benzene for 6 hr/day on days 6 to 15 of gestation (*110*). Significantly increased incidences of missing sternebrae were observed in the female offspring after exposures of the mothers at 2200 ppm. Delayed ossification was also found in the fetuses of Sprague-Dawley rats exposed to air containing 50 or 500 ppm benzene, 7 hr/day on days 6 to 15 of gestation. The mean number of caudals (vertebrae) in the fetuses of the rats that were exposed was significantly less than that in the fetuses of controls (*111*).

Skeletal retardation and abnormalities (but not malformations) were observed in the offspring of CFY rats exposed to air containing 313 ppm benzene, 24 hr/day on days 9 to 14 of gestation (*112*). Fetal weight retardation, resorptions, fetal deaths, and skeletal variants but no skeletal malformations, were observed following inhalation exposure of pregnant CFLP mice to 500 or 1000 mg/m^3 (156 or 313 ppm) benzene on days 7 to 20 of gestation; New Zealand rabbits showed maternal and fetal weight reductions and abortions at 313 ppm, but not at 156 ppm (*113*). Coate et al. (*114*) reported fetal weight loss in Sprague-Dawley rats at 100 ppm benzene but not at 1, 10, or 40 ppm. In CFY rats exposed to 125 ppm benzene, maternal and fetal weights were reduced and skeletal variants were increased (*115*).

Keller et al. and Keller and Snyder (*116,117*) documented alterations in fetal and offspring hematopoiesis following exposure of pregnant Swiss-Webster mice to 5, 10, or 20 ppm benzene during days 6 to 15 of gestation. Ward et al. (*118*) reported that CD-1 mice exposed to 300 ppm benzene for 13 weeks exhibited ovarian cysts, testicular degeneration, atrophy, and decreased spermatozoa; animals exposed to 1, 10, or 30 ppm benzene vapor showed no adverse effects.

No teratogenic effects were observed in offspring following 6-hr daily exposure of pregnant Sprague-Dawley rats to benzene vapor at 0, 10, 40, or 100 ppm on days 6 to 15 of gestation (*119*). A slight fetotoxic effect (reduced mean fetal body weights) was reported for both sexes of offspring of the mothers that were exposed to benzene at 100 ppm. The available data on teratologic studies have been summarized (*120,121*). Schwetz (*120*) concluded that benzene has not been found to be teratogenic in laboratory animals exposed during the critical period of development of the embryo or fetus. After review of the teratogenic studies, the author stated that exposure to benzene at levels that do not cause other forms of toxicity would not be expected to cause adverse development effects. Davis (*122*) reviewed the literature on the reproductive risks of benzene and concluded that more animal and epidemiologic studies are needed before definitive conclusions can be made.

Carcinogenesis Studies on Benzene Metabolites

The many available long-term carcinogenicity studies on benzene *per se* as conducted and reported by various investigators are not specifically discussed or summarized in our paper. These have been reviewed (*123*) and evaluated (*124*). Further, Cronkite et al. (*125*) and Maltoni et al. (*126*) report on their numerous studies as well as record much of the carcinogenesis literature on benzene. Rather, because several metabolites of benzene have been studied in long-term carcinogenesis bioassays, we recapitulate the available studies on phenol, catechol, hydroquinone, and *p*-quinone in an attempt to give a broader perspective to the results observed after benzene exposure alone.

Phenol

Carcinogenesis studies of phenol (CAS No. 108-92-2) were conducted by providing drinking water containing 0, 2500, or 5000 ppm to F344 rats and to $B6C3F_1$ mice for 103 weeks (*127,128*). Increased incidences of three neo-

plasms were detected in low-dose male rats [leukemia, 18/50, 30/50 ($p < 0.05$), 25/50; pheochromocytoma of the adrenal glands, 13/50, 22/50 ($p < 0.05$], 9/50; and C-cell carcinoma of the thyroid gland, 0/50, 5/49 ($p < 0.05$), 1/50]. No chemical-related neoplasms were observed in female rats or in male and female mice. Several investigators have shown that phenol in acetone or in benzene promotes skin cancer in mice pretreated with 7,12-dimethylbenz[a]anthracene (DMBA) or 3,4-benzo[a]pyrene (BaP) (*129–133*). Phenol did not exhibit any cocarcinogenic effects when given with BaP (*133,134*).

Catechol

In 1951, Lehman et al. (*135*) reported a study in which groups of 12 to 18 rats were exposed to 0.0625 to 1.0% catechol (CAS No. 120-80-9) in the diet for 2 years. The only response recorded was "beginning hepatic cell hyperplasia" in the 0.25% group. In a short-term skin tumor-promoting study, 30 female Sutter mice received 0.3 mL DMBA in benzene and, beginning 1 week later for 15 weeks, a single drop of 15% catechol in benzene. No promoter activity was observed (*130*). Van Duuren and Goldschmidt applied 150 μg BaP topically to the dorsal skin of 50 female Swiss mice and 14 days later applied 2 mg catechol in 0.1 mL acetone three times per week until day 448 (*133*). No tumor-promoting activity was found. Hecht et al. (*136*) applied 75 μg DMBA in 0.1 mL acetone to groups of 30 female Swiss mice one time only, followed 10 days later with 0.1 mL of a 1% catechol/acetone (1 mg of catechol) solution five times per week for 67 weeks. In these studies, catechol was inactive as a tumor promoter. Van Duuren et al. (*134*) and Van Duuren and Goldschmidt (*133*) applied 2 mg catechol with and without 5 μg BaP in 0.1 mL acetone to groups of 50 female Swiss mice three times per week for 368 days. Of the mice dosed with catechol and BaP in combination, 36 had skin papillomas (2.5 per mouse) and 31 had skin squamous cell carcinomas. In the group receiving only catechol, one mouse had a skin papilloma and a squamous cell carcinoma. Thirteen mice receiving BaP had skin papillomas (1.1 per mouse), and 10 had squamous cell carcinomas. In these experiments, catechol increased the carcinogenic activity of BaP. Subsequently, Hecht et al. (*137*) tested a subfraction of cigarette smoke condensate containing 97% catechol on groups of 30 female Swiss mice. A 0.25% catechol-acetone solution (0.1 mL) alone or with 0.003% BaP was applied five times per week for 52 weeks [this dose was about one-sixth the dose used by Van Duuren and Goldschmidt (*133*)]. The combination caused increased incidences of neoplasms per mouse (1.4 versus 0.1) and greater percentages of mice with skin tumors (73% versus 14%) and squamous cell carcinomas (64% versus 11%). This study confirms that catechol possesses cocarcinogenic effects. Boyland et al. (*138*) implanted 10-mg cholesterol pellets alone or containing 20% catechol into the urinary bladders of mice. Of the 19 mice that received the combination pellet and survived the 25-week experiment, 1 (5.3%) had a papilloma and 3 (15.8%) had carcinomas. Of 77 mice with cholesterol pellets, 4 (5%) had adenomas or papillomas and 5 (6.5%) had carcinomas. A marginal decrease ($p = 0.03$) was observed for the catechol group (4/19, 21.1% versus 9/77, 11.7%).

In an oral exposure study lasting 52 weeks, catechol caused adenomatous hyperplasia and adenocarcinomas of the glandular stomach and hyperplasia of the forestomach in F344 male rats (*139*). Hirose et al. (*139*) exposed groups of 10 to 20 male rats to a) *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) alone (a single intragastric dose of 150 mg/kg body weight), b) MNNG followed 1 week later by catechol in feed (1.5% in the diet for 4 weeks, then reduced to 0.8% for 47 weeks due to lack of body weight gain), c) catechol alone, and d) basal diet alone. The histopathology findings are given in Table 1. These data show that catechol (after MNNG) strongly enhanced both forestomach and glandular stomach carcinogenesis. Catechol alone caused cancer in the pyloric region of the glandular stomach. Thus catechol appears to be one of the few environmental chemicals shown to cause cancer of the glandular stomach.

Hydroquinone

Lehman et al. (*135*) fed hydroquinone (CAS No. 123-31-9) at 0.125 to 2.0% in the diet to groups of 12 to 18 rats for 2 years. The authors reported a "suggestion" of gastrointestinal ulceration and renal tumors in the 2% group. Carlson and Brewer (*140*) offered diets containing 0 to

Table 1. Neoplastic and preneoplastic lesions in the forestomach and glandular stomach epithelium of rats treated with MNNG followed by catechol.[a]

		No. of rats with lesions, %							
		Forestomach				Fundus		Pylorus	
Chemical treatment	No. of rats[b]	Hyperplasia	Papilloma	Carcinoma *in situ*	Squamous cell carcinoma	Adenomatous hyperplasia	Adeno-carcinoma	Adenomatous hyperplasia	Adeno-carcinoma
MNNG	19	19 (100)	13 (68)	11 (60)	5 (26)	0	0	1 (5)	0
MNNG, then catechol	19	19 (100)	18 (95)	8 (42)	19 (100)*	3 (16)	1 (5)	19 (100)*	18 (95)*†
Catechol	15	13 (87)	1 (7)	0	0	0	0	15 (100)‡	3 (20)
Basal diet	10	0	0	0	0	0	0	0	0

[a]From Hirose et al. (*139*).
[b]Surviving at the end of experiment.
*$p < 0.001$ vs. control group (MNNG alone).
†$p < 0.001$ vs. control group (catechol alone).
‡$p < 0.001$ vs. control group (basal diet alone).

0.5% or 0 to 1.0% (four concentrations) hydroquinone with 0.1% citric acid to groups of 10 to 23 male and female rats for 103 weeks. No adverse hematologic or histopathologic effects were recorded. Roe and Salaman (*141*) applied 0.3 mL of a 6.7% hydroquinone-acetone solution (20 mg dose) to the backs of male *S*-strain mice. Three weeks later croton oil (0.3 mL of a 0.5% acetone solution) was applied dermally once a week for 18 weeks. One of the 22 survivors had a skin papilloma. Hydroquinone was inactive as an initiator of skin carcinogenesis.

Using experimental conditions similar to those described above for catechol, Boutwell and Bosch (*130*) and Van Duuren and Goldschmidt (*133*) reported that hydroquinone had no promoter activity with DMBA or BaP. Boyland et al. (*138*) tested hydroquinone as 20% in 10-mg cholesterol pellets implanted in the urinary bladder of mice. Six of the 19 survivors (32%) at week 25 had bladder carcinomas, whereas 4 benign and 5 malignant neoplasms were found in 77 cholesterol controls (11.7%). In this study, hydroquinone was considered carcinogenic.

In carcinogenesis studies, Van Duuren and Goldschmidt (*133*) applied hydroquinone in doses of 5 mg with and without 5 μg BaP in acetone. Hydroquinone with BaP induced fewer skin neoplasms than BaP alone (7 mice with 11 papillomas and 3 with squamous cell carcinomas versus 14 mice with 16 papillomas and 10 with squamous cell carcinomas).

Two-year carcinogenesis studies of hydroquinone have been conducted in male and female F344/N rats and B6C3F$_1$ mice (*142*). Hydroquinone was given in water by gavage at doses of 0, 25, or 50 mg/kg for rats and 0, 50, or 100 mg/kg for mice. Under the conditions of these experiments, hydroquinone caused papillary hyperplasia of the transitional epithelium, cysts, and tubular cell adenomas (0/55 controls, 4/55 low dose, 8/55 top dose) of the kidney in male rats; tubular cell hyperplasia was seen in two additional high-dose rats. Pheochromocytomas of the adrenal gland were somewhat increased (14/55, 19/48, 21/55), yet these lesions were not considered related to chemical exposure. In female rats, mononuclear cell leukemia was observed at higher incidences in exposed groups (9/55, 15/55, 22/55). For mice, hydroquinone induced neoplasms of the liver in female mice (mainly adenomas: 2/55, 15/55, 12/55; also, carcinomas were observed in 1 control, 2 low-dose, and 2 top-dose female mice), and in both sexes follicular cell hyperplasia of the thyroid gland was increased (male: 5/55, 15/53, 19/54; female: 13/55, 47/55, 45/55). In female mice a marginal increase in follicular cell adenomas of the thyroid gland (3/55, 5/55, 6/55) was observed [the background incidence of these tumors is 2.1% (41/1937) versus 5% in controls, 9% in low dose, and 11% in top dose]. A follicular cell carcinoma of the thyroid gland was found in another top dose female mouse.

p-Quinone

Three carcinogenesis studies have been reported for *p*-quinone (CAS No. 106-51-4) (*143*). In 1940, Takizawa painted the skin of mice every 1 or 2 days for about 200 days with 0% (benzene only), 0.1%, or 0.25% *p*-benzoquinone in benzene. Of 46 controls, 1 had a skin papilloma and 2 had adenocarcinomas of the lung. Among the 41 mice at 0.1%, 6 had skin papillomas, 2 had skin carcinomas, and 10 developed lung adenocarcinomas. In the 0.25% group (44 mice), 3 had skin papillomas, 1 had a skin carcinoma and 5 had lung adenocarcinomas. In a series of three inhalation experiments, Kishizawa (*144-146*) exposed groups of 25 mice to air containing 0 or 5 mg *p*-quinone, once per day, six times per week. The numbers of mice with neoplasms of the lung were not different between control and dosed groups. Umeda (*147*) conducted studies in 24 rats by injecting 0.5 mL of propylene glycol with *p*-quinone SC once per week for 394 days (concentrations were 1% from days 1 to 53, 0.2% from days 54 to 173, and 0.4% from days 173 to 394). Of 17 surviving rats receiving 32 injections (81 mg *p*-quinone and 16.5 mL propylene glycol), 2 developed injection-site fibrosarcomas.

Akyl Benzenes

We have also evaluated in long-term carcinogenicity studies the two simple alkylbenzenes: methyl benzene (toluene) and dimethyl benzenes (mixed xylenes). The mixed xylenes (composed of 9% 1,2-; 60% 1,3-; 14% 1,4-xylene; and 17% ethylbenzene) were given to F344 rats (0, 250, 500 mg/kg) and to B6C3F$_1$ mice (0, 500, 1000 mg/kg) by oral intubation. No evidence of carcinogenicity was found in male rats, female rats, male mice, or female mice exposed 5 days/week for 2 years (*2,148*). In the toluene studies F344 rats and B6C3F$_1$ mice were exposed by inhalation (rats: 0, 600, 1200 ppm; mice: 0, 120, 600, 1200 ppm) 6.5 hr/day, 5 days/week, for 2 years (*2,149*). The only interesting findings from this study are that tubular cell adenomas of the kidney were found in one low-dose and in two top-dose male rats, and another low-dose male rat had a renal transitional epithelial carcinoma; one top-dose female rat had a renal tubular cell carcinoma. These lesions were not supported by the occurrence of tubular cell hyperplasia. None were observed in controls or in male or female mice. Because the kidney is a target organ (severity of nephropathy was increased), because the structural analogues benzene and xylene did not cause renal tumors and because the finding of these few uncommon tumor types were possible signals for potential public health concern, we decided to evaluate in extra detail this putative neoplastic target organ in an attempt to get closer to the true incidence of these lesions. For each male rat an additional six tissue sections were evaluated; thus for each group of 60 rats, 360 sections (total of 1080 sections) were prepared and read. The results of this supplementary study revealed nine microscopic adenomas that were not discovered on routine sectioning—five in controls and four in the low-dose group. All 12 tubular cell neoplasms were benign. The combined diagnoses allows the more confident conclusion that toluene did not cause any increases in hyperplasia, benign neoplasia, or malignant neoplasms of the kidney.

Likewise neither benzene nor xylene was associated with kidney toxicity.

Effects on Humans

Humans are susceptible to benzene myelotoxicity as shown by the high incidence of pancytopenia among workers with significant exposure (*150*). The connection between myelotoxicity and acute myelogenous leukemia remains a subject of considerable discussion. Blood diseases associated with benzene exposure include pancytopenia (most frequently cited), leukopenia, bone marrow hypoplasia or aplasia, thrombocytopenia, granulocytopenia, and lymphocytopenia (*11,25,151-153*). Benzene, a myelotoxic chemical, causes pancytopenia and eventual aplastic anemia in most animal species exposed.

Ever since Santesson in 1897 (*154*) and Selling in 1916 (*14*) recorded that benzene could cause aplastic anemia, numerous supporting reports have been published (*11,124*). The correlation of exposure levels to specific hematologic toxicity has been well documented; however, it is not possible to reliably predict effects produced at specific exposure levels. Likewise, it has not been possible to establish with certainty the degree of exposure below which no adverse hematologic effects in humans would occur.

Effects in humans from benzene exposure have been well characterized and described in medical, toxicology, and poisoning treatment manuals and texts. Although the benzene-associated signs and symptoms in humans cannot be related exactly or with critical accuracy, the biologic events that occur in humans from increasing relatively short-term exposure to benzene appear to follow a pattern from effects on the hematopoietic system, through narcosis, and death. Loss of consciousness, irregular heartbeat, dizziness, headache, and nausea were observed in workers exposed to benzene at concentrations below 20,000 ppm (*155*). Reports that single exposures at concentrations of 20,000 ppm were fatal within 5 to 10 min have been made (*156*). Continued exposure of workers to benzene has been associated with decreased concentrations of circulating erythrocytes, leukocytes, and thrombocytes (*157*). The incidence of sister-chromatid exchanges was not significantly increased in the lymphocytes of 22 workers in Italy exposed to benzene at 0.2 to 12.4 ppm (mean exposure time, 11.4 years) in air as compared with persons living in the same area of similar age and smoking habits (*158*). The incidence of chromosome-type aberrations was significantly greater among exposed workers compared with controls.

Although a link between benzene exposure and hematologic disorders was suggested 80 years ago, a connection with leukemia was not clearly established at that time. In early epidemiologic studies, workers were exposed to other chemicals in addition to benzene (*11,25,124,149,151*).

An association between long-term exposure to benzene and the occurrence of leukemia was suggested as early as 1928 by Delore and Borgomano (*159*), who described acute lymphoblastic leukemia in a worker exposed to benzene for 5 years. IARC (*11,124*) gives a chronology of published literature. Goldstein (*160*) describes a number of additional case reports. Most malignancies in which an association with exposure to benzene has been reported have been leukemias, particularly those of the myelogenous type. A critical issue in benzene risk assessment seems to center on the interpretation of the shape associated with the dose-response curve relating benzene exposure to acute myelogenous leukemia and variants.

More than 100 occurrences of leukemia in humans have been associated with benzene exposure since 1928 (*159,161*). More recent epidemiologic studies of small cohorts exposed to benzene have demonstrated a causal association for leukemia (*11,20,23,25,124,162-169*). Rinsky et al. (*163*) examined the updated mortality of a cohort with occupational exposure to benzene and calculated a cumulative benzene exposure index (parts per million × years) for each cohort member. These authors found that the standard mortality ratio (SMR) for leukemia was 328 and for multiple myeloma was 398. With stratification of the cohort by cumulative exposure, the SMRs for leukemia increased from 105 in workers with less than 40 ppm-years of exposure to 314 in workers with 40 to 199 ppm-years, to 1757 in those with from 200 to 399 ppm-years, and to 4535 in those with 400 ppm-years or more. Most of these studies are summarized in the December 1985 and September 1987 Federal Register notices on occupational exposure to benzene (*23,25*) and to a lesser degree by Austin et al. (*170*), Goldstein (*171*), and IARC (*124*).

After evaluating the available published data IARC (*11,124*) considered benzene to be a Group 1 carcinogen: sufficient evidence of carcinogenicity to humans, and as having sufficient evidence of carcinogenicity in laboratory animals. More current data on humans are presented in these proceedings by Aksoy (*168*) for a large cohort of Istanbul shoe workers and by Yin et al. (*169*) for a large cohort of factory workers in China; the latter authors found that for males the standardized mortality ratios were elevated for leukemia, lung cancer, liver cancer, and stomach cancer. And for females an excess in leukemia was observed among the exposed. Given the overwhelming and conclusive carcinogenicity findings in laboratory animals (*1,2,11,123-126,188,189*), Huff (*25*) testified that the consistency and magnitude of the responses in animals at relatively low exposures led him to the obvious speculation that there would likely be other types of cancers as well as leukemias that one would suspect to occur in humans. Yin et al. (*169*) has unfortunately shown this to be true.

Study Rationale

Benzene was studied for long-term effects in rodents by the NTP Carcinogenesis Program because of its large production volume, because of epidemiologic evidence that exposure of humans to benzene is associated with an increased incidence of leukemia, and because previous experimental studies for carcinogenicity in laboratory animals were considered to be inconclusive or inadequate.

In the studies reported in this paper, benzene was given by gavage in corn oil because the chemical was only slightly soluble in water, because benzene was considered too volatile to be administered by feed, and to make certain that the animals would be exposed to a sufficient and accurately calculated amount of the chemical. In retrospect, the inhalation route may have been a more appropriate mimic of the major route of human exposure.

Nonetheless there is no convincing evidence that the oral route used in these and other experiments would be other than applicable and relevant to humans.

Materials and Methods

Procurement and Characterization of Benzene

Benzene was obtained from Burdick and Jackson Laboratories (Muskegon, MI). Lot numbers AB223 and AB490 were used for both the 17-week and 2-year studies. A comparison of the two lots by gas chromatography indicated both were similar. Thereafter, lot no. AB223 was used as a reference to identify both lots.

Purity and identity analyses were conducted at Midwest Research Institute (Kansas City, MO). Results of elemental analyses for carbon and hydrogen agreed with the theoretical values. Four impurities with a total area 0.2% that of the major peak were detected by gas chromatography in one system. Five impurities with a total area less than 0.90% that of the major peak were detected by a second gas chromatographic system. The infrared, ultraviolet/visible, and nuclear magnetic resonance spectra were consistent with those in the literature. Benzene was stored in its original container at room temperature. Results of periodic analyses of the bulk chemical by gas chromatography and infrared spectroscopy indicated that the chemical was stable throughout these studies. The benzene used in these studies was greater than 99.7% pure.

Preparation and Characterization of Dose Mixtures

A weighed amount of benzene was mixed with the appropriate amount of Mazola corn oil and mixed by inversion. Benzene in corn oil was found to be stable at 25°C for at least 7 days (Table 2). Dose mixtures were routinely used within 2 weeks of preparation. All benzene/corn oil mixtures analyzed were within ± 10% of the target concentrations (Table 3).

Seventeen-Week Studies

Seventeen-week studies were conducted to evaluate the cumulative toxicity of benzene, to identify target organs, and to determine the doses to be used in the 2-year studies. Four-week-old F344/N rats and 6-week-old $B6C3F_1$ mice of each sex were obtained from Charles River Breeding Laboratories, observed for 15 days, and then assigned to cages according to a table of random numbers. Cages were then assigned to vehicle control and dosed groups according to another table of random numbers.

Groups of 10 rats and 10 mice of each sex were administered 0, 25, 50, 100, or 400 mg/kg benzene in corn oil by gavage, 5 days per week for 17 weeks. Groups of 15 rats and 15 mice of each sex were administered 0, 200, or 600 mg/kg. Rats and mice were housed 5 per cage. Feed and water were freely available. Animals were checked twice daily; moribund animals were killed. Animal weights were recorded weekly.

Hematologic analyses were performed on blood taken from the orbital sinuses of five rats and five mice of each sex killed on day 0 and on day 60, of five rats and five mice of each sex from the 0, 200, and 600 mg/kg groups. At the end of the 120-day studies, survivors were killed and hematologic analyses were performed on five animals from each group. A necropsy was performed on all animals.

Table 2. Stability of benzene in corn oil.

Storage time, days	Average % of benzene found in benzene vehicle mixture[a,b]
1	3.78 ± 0.04[c]
4	3.80 ± 0.04
5	3.83 ± 0.04
7	3.82 ± 0.04

[a]Zero-time recovery yield, 100.0% ± 0.9%.
[b]Target concentration of chemical in corn oil, 3.83% ± 0.02%.
[c]Values are means ± SD.

Table 3. Analysis of dose mixtures in the 2-year gavage studies of benzene.

	Concentration of benzene in corn oil for target concentration, mg/mL[a]			
Weeks on study	5	10	20	40
0	5.4	9.5	19.1	38.2
07	4.8	10.9	21.6	39.3
15	5.1	10.1	20.2	38.0
26	5.0	10.9	20.6	40.5
31	5.4	11.0	21.4	41.7
40	4.7	9.8	20.4	40.9
48	5.1	10.6	20.1	37.1
57	5.2	10.3	20.7	41.3
64	5.5	10.3	21.4	37.5
72	5.4	10.7	21.1	37.2
78	5.4	10.9	19.8	38.9
88	5.2	10.7	21.2	41.5
96	5.2	10.0	20.2	41.0
104	4.6	9.4	20.4	—
Mean mg/mL	5.1	10.4	20.6	39.5
SD	0.28	0.54	0.70	1.75
Coefficient of variation, %	5.5	5.2	3.4	4.4
Range, mg/mL	4.6-5.5	9.4-11.0	19.1-21.6	37.1-41.7

[a]Results of duplicate analysis.

Two-Year Studies

Study Design. Groups of 60 male rats were administered 0, 50, 100, or 200 mg/kg benzene in corn oil by gavage, 5 days/week for 103 weeks. Groups of 60 female rats and 60 mice of each sex were administered 0, 25, 50,

or 100 mg/kg. Blood was withdrawn from 10 randomly preselected animals from each sex and dose group (nos. 41-50) at 12, 15, 18, and 21 months. Blood was also taken from moribund animals before they were killed and from all animals at the end of the experiment at 24 months. Groups of 10 animals of each sex and species from both control and exposed groups were removed at 51 weeks at the doses of the 2-year studies; blood was withdrawn at 0, 3, 6, 9, and 12 months, and at 12 months these animals were killed and necropsies were performed.

Hematologic Analyses. Hematologic analyses included packed cell volume, red blood cell count, total and differential white blood cell count, hemoglobin, and mean corpuscular volume. Reticulocyte count and prothrombin time (PRT) were also determined.

Source and Specifications of Animals. The male and female F344/N rats and B6C3F$_1$ (C57BL/6N, female, × C3H/HeN MTV$^-$, male) mice used in this study were produced under strict barrier conditions at the Charles River Breeding Laboratories (Portage, MI) under a contract to the Carcinogenesis Program. Breeding stock for the foundation colonies at the production facility originated at the National Institutes of Health Repository. Animals shipped for study were progeny of defined microflora-associated parents that were transferred from isolators to barrier-maintained rooms. Animals were shipped to the laboratory at 4 to 5 weeks of age. The animals were quarantined at the facility for 2 weeks. Thereafter, a complete necropsy was performed on five animals of each sex and species to assess their health status. The rats were placed on study at 7 to 8 weeks of age and the mice at 6.5 to 8.5 weeks of age. The health of the animals was monitored during the course of the study according to the protocols of the NTP Sentinel Animal Program.

A quality control skin grafting program has been in effect since early 1978 to monitor the genetic integrity of the inbred mice used to produce the hybrid B6C3F$_1$ animal. In mid-1981, data were obtained that showed incompatibility between the NIH C3H reference colony and the C3H colony from a Program supplier. In August 1981, inbred parental lines of mice were further tested for genetic integrity via isozyme and protein electrophoresis profiles that demonstrate phenotype expressions of known genetic loci. The C57BL/6 mice were homogeneous at all loci tested. Eighty-five percent of the C3H mice monitored were variant at one to three loci, indicating some heterogeneity in the C3H line from this supplier. Nevertheless, the genome of this line is more homogeneous than that of randomly bred stocks. Further, all animals used in these studies were genetically identical. Male mice from the C3H colony and female mice from the C57BL/6 colony were used as parents for the hybrid B6C3F$_1$ mice used in these studies. The influence of the potential genetic nonuniformity in the hybrid mice on these results is not known, but results of the studies are not affected because concurrent controls were included in each study.

Animal Maintenance. Animals were housed five per cage. Feed and water were freely available.

Clinical Examinations and Pathology. All animals were observed twice per day, and clinical signs were recorded once per week. Body weights by cage were recorded once per week for the first 12 weeks of the study and once every 4 weeks thereafter. Mean body weights were calculated for each group. Moribund animals were killed, as were animals that survived to the end of the study. A necropsy was performed on all animals, including those found dead.

Examinations for grossly visible lesions were performed on major tissues or organs. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, placed on slides, stained with hematoxylin and eosin, and evaluated.

When the pathology examination was completed, the slides, individual animal data records, and summary tables were sent to an independent quality assurance laboratory. Individual animal records and tables were compared for accuracy, slides and tissue counts were verified, and histotechnique was evaluated. All tumor diagnoses, all target tissues, and all tissues from a randomly selected 10% of the animals were evaluated by a quality assurance pathologist. Slides of all target tissues and those about which the original and quality assurance pathologists disagreed were submitted to the Pathology Working Group (PWG) made up of six pathologists for evaluation. Representative coded slides selected by the Chairperson were reviewed by PWG pathologists, who reached a consensus and compared their findings with the original and quality assurance diagnoses. When diagnostic differences were found, the PWG sent the appropriate slides and comments to the original pathologist for review. This procedure has been described, in part, by Maronpot and Boorman (*172*) and Boorman et al. (*173*). The final diagnoses represent a consensus of study and quality assurance pathologists and the NTP Pathology Working Group. For subsequent evaluations, the diagnosed lesions for each tissue type are combined according to the guidelines of McConnell et al. (*174*).

Statistical Methods

Survival Analysis. The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (*175*). Statistical analyses for a possible dose-related effect on survival used the methods of Cox (*176*) and Tarone (*177*).

Calculation of Incidence. The incidence of neoplastic or nonneoplastic lesions is given as the ratio of the number of animals bearing such lesions at a specific anatomic site to the number of animals in which that site was examined.

Analysis of Tumor Incidence. Three statistical methods are used to analyze tumor incidence data. Tests of significance included pairwise comparisons of high-dose and low-dose groups with vehicle controls and tests for overall dose-response trends. For studies in which compound administration has little effect on survival, the results of the three alternative analyses will generally be similar. When differing results are obtained by the three

methods, the final interpretation of the data will depend on the extent to which the tumor under consideration is regarded as being the cause of death. All reported p-values for tumor analyses are one-sided.

Life table analyses assume that all tumors of a given type observed in animals dying before the end of the study were fatal; i.e., they either directly or indirectly caused the death of the animal. The underlying variable considered by this analysis is time to death due to tumor. If the tumor is rapidly lethal, then time to death due to tumor closely approximates time to tumor onset.

Incidental tumor analyses assume that all tumors of a given type observed in animals that died before the end of the study were incidental, i.e., they were merely observed at necropsy in animals dying of an unrelated cause.

Primarily, the two survived-adjusted methods discussed are used to evaluate tumor incidence. The Fisher exact test for pairwise comparisons and the Cochran-Armitage linear trend test (*178,179*) are based on the overall proportion of tumor-bearing animals and do not adjust for survival differences. For details concerning the statistical analysis of tumor incidence, see Haseman (*180*).

Hematology data were analyzed by a repeated measures analysis of variance (*181*). Pairwise comparisons were made by the Dunnett multiple comparisons test (*182,183*).

Results

Seventeen-Week Studies

No compound-related deaths occurred in rats. Final mean body weights (relative to those of the vehicle controls) were depressed 14 to 22% for male and female rats that received 200, 400, or 600 mg/kg benzene.

A dose-related leukopenia was observed for both male and female rats. Lymphoid depletion in the spleen was observed in 3/5 male and 4/5 female rats that received 200 mg/kg benzene and 5/5 male and 5/5 female rats that received 600 mg/kg benzene for 60 days and in 10/10 male and 10/10 female rats that received 600 mg/kg for 120 days. Increased extramedullary hematopoiesis was observed in the spleen of 4/5 male and 3/5 female rats that received 600 mg/kg for 120 days. Based on these composite observations, doses selected for rats for the 2-year studies were 0, 50, 100, or 200 mg/kg benzene in corn oil for males and 0, 25, 50, or 100 mg/kg for females.

No compound-related deaths occurred in mice. Final mean body weights (relative to those of the vehicle controls) were depressed 4 to 10% for all dosed groups that received 100 mg/kg or more of benzene. Tremors were observed intermittently in the 400 and 600 mg/kg groups throughout the studies, and during the last 3 weeks of the studies, tremors were more pronounced in male mice than in females. A dose-related leukopenia was observed for both male and female mice. No compound-related histopathologic effects were observed. Based on these findings, doses selected for mice for the 2-year studies were 0, 25, 50, and 100 mg/kg benzene in corn oil.

Two-Year Studies

Body Weights and Clinical Signs. Dose-related weight gain reduction, with earlier and more pronounced differences, occurred in male rats (Figs. 1 and 2). After week 22, mean body weights of dosed male rats were lower than those of the vehicle controls; the 200 mg/kg group had body weights that were 11% lower than vehicle controls at week 25 and that continued to be lower until the difference was 23% at week 103. The low-dose group was similar to vehicle controls throughout the study; the mid-dose group showed about a 7 to 9% lower body weight after 1 year. After week 62, mean body weights of high-dose female rats were somewhat lower than those of the vehicle controls. Only the top-dose group showed reductions greater than 5% and the maximum difference (−9.5%) occurred at week 103.

Other than modest decreases in body weights in each group compared with vehicle controls (except for the 200 mg/kg male rats), no extraordinary clinical signs were recorded. Nonspecific observations included a varying degree of ocular discharge. This common syndrome in F344 rats was first observed at 4 to 5 months in both sexes and across groups. A serous discharge varying from clear to red-tinged or yellow and from scant to heavy occurred in about 10% of all rats; this may have been associated with blood sampling techniques. Near the end of the studies, a paleness of mucosa was recorded for rats in a moribund state before death.

Mean body weights of mice were variable after week 45, and for high-dose male mice were lower than those of the vehicle controls after week 47, ending in a difference of −19% at 103 weeks (Fig. 3). Mean body weights for low- and mid-dose groups were like those of vehicle controls until about the final 4 or 15 weeks of the study. Mean body weights of vehicle control and high-dose female mice were comparable until week 87. From week 87 to the end of the study, mean body weights of high-dose female mice were lower than those of the vehicle controls; at week 103, the reduction was 15% (Fig. 4). No compound-related clinical signs were observed.

Survival. Estimates of the probabilities of the survival of male and female rats administered benzene at the doses used in these studies and those of the vehicle controls are shown in the Kaplan and Meier (*175*) curves in Figures 5 and 6. The survival of the high-dose group of male rats was significantly lower than that of the vehicle control group after week 95 (Table 4). In females, the survival of both the mid-dose group after week 101 and high-dose groups after week 96 was significantly lower than that of the vehicle control group; this latter group had exceptionally good survival (92%) compared with the historical rate of 74% ± 7% (SD) (*184*).

Estimates of the probabilities of survival for male and female mice administered benzene at the doses used in these studies and for the vehicle controls are shown in the Kaplan and Meier curves in Figures 7 and 8. The survival of the high-dose group of both male mice after week 96 and female mice after week 92 was significantly lower than that of the respective vehicle control groups (Table 5).

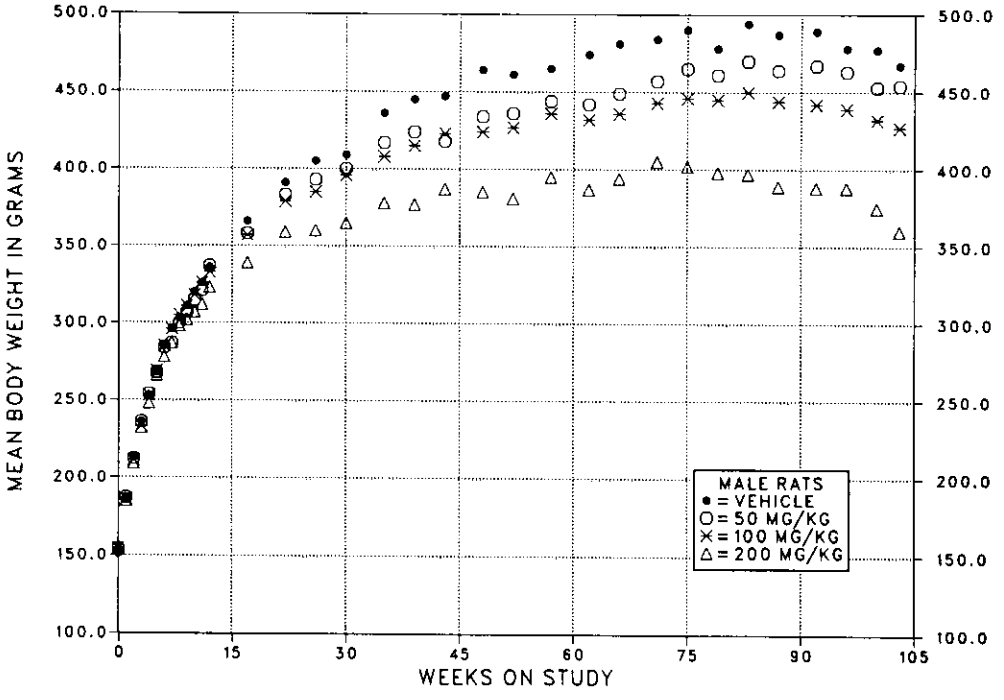

FIGURE 1. Kaplan-Meier survival curves for male rats administered benzene in corn oil by gavage for 2 years.

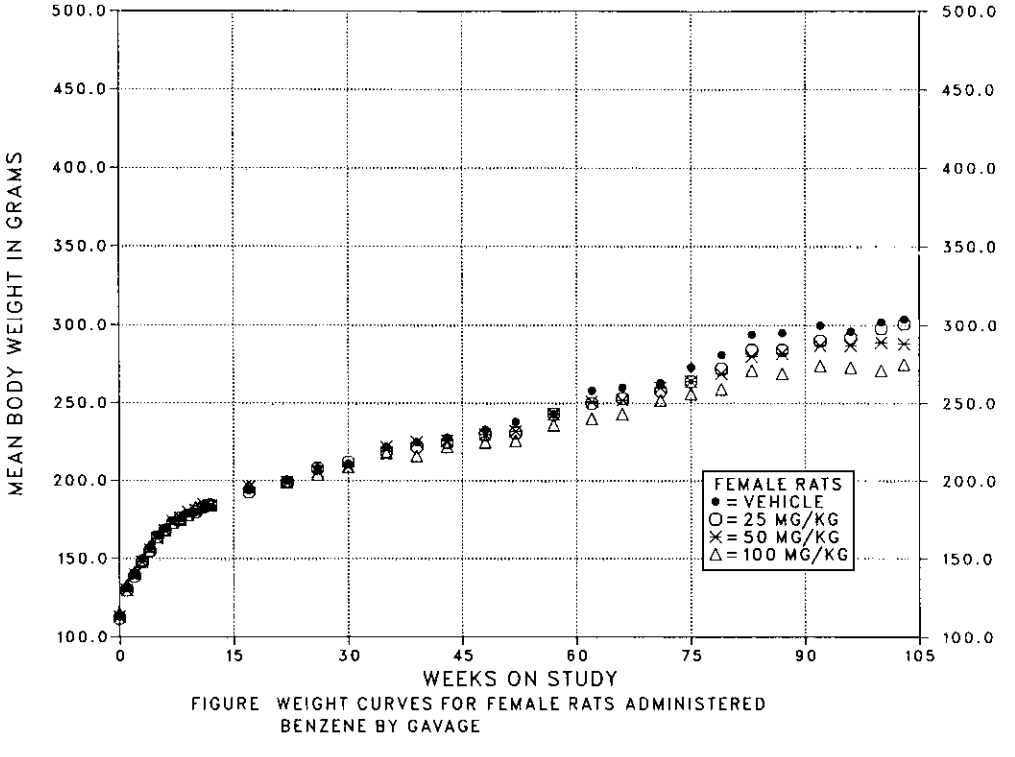

FIGURE 2. Growth curves for female rats administered benzene in corn oil by gavage for 2 years.

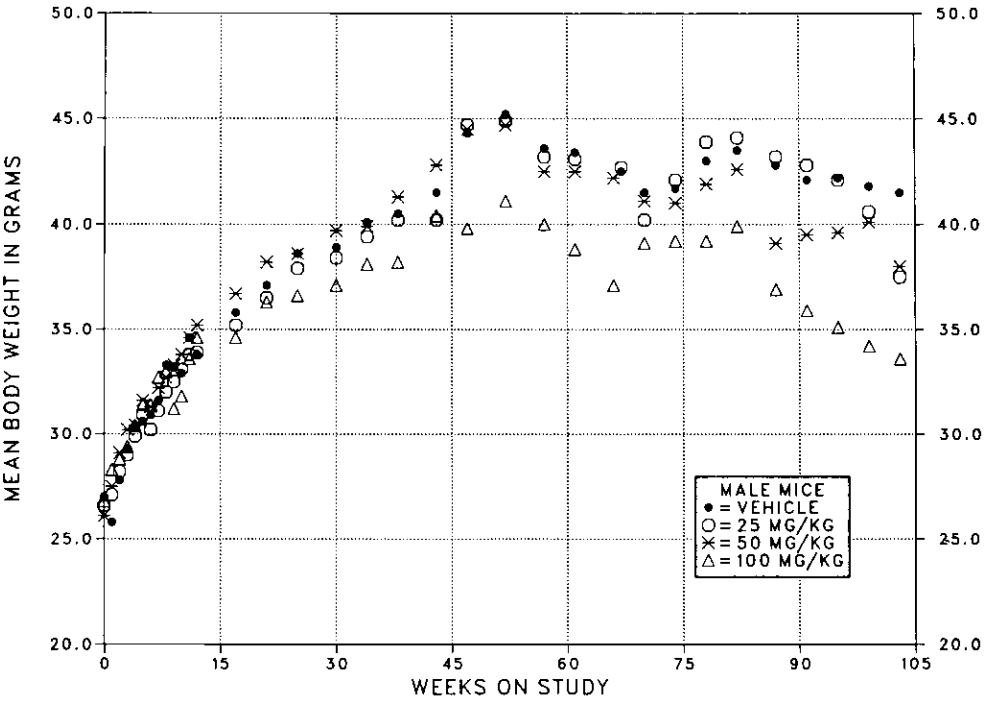

FIGURE 3. Growth curves for male mice administered benzene in corn oil by gavage for 2 years.

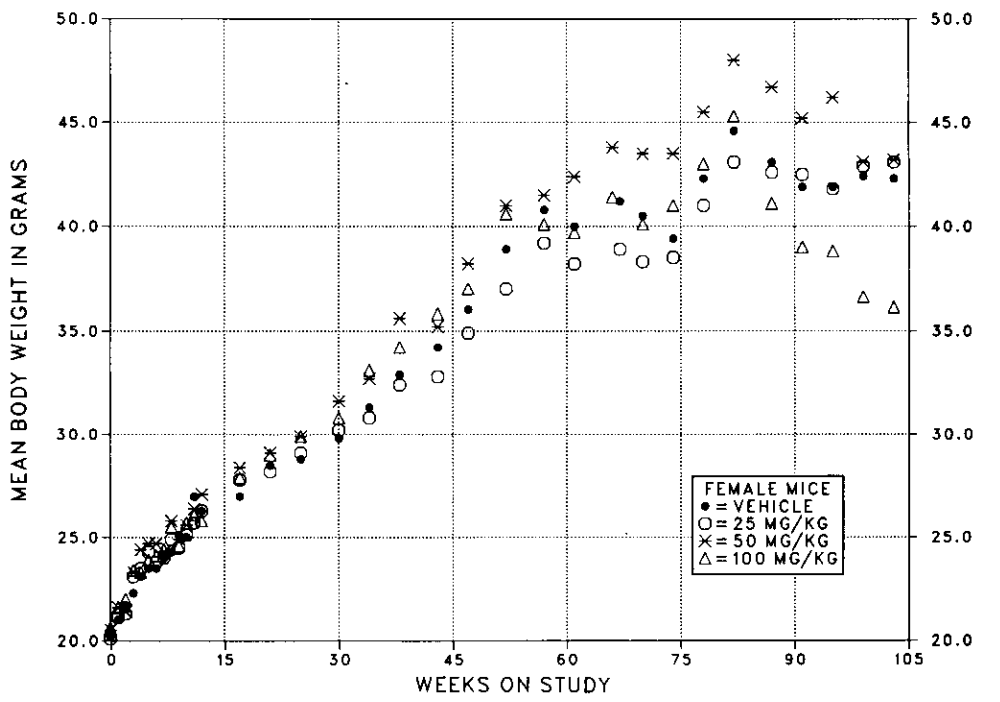

FIGURE 4. Kaplan-Meier survival curves for female mice administered benzene in corn oil by gavage for 2 years.

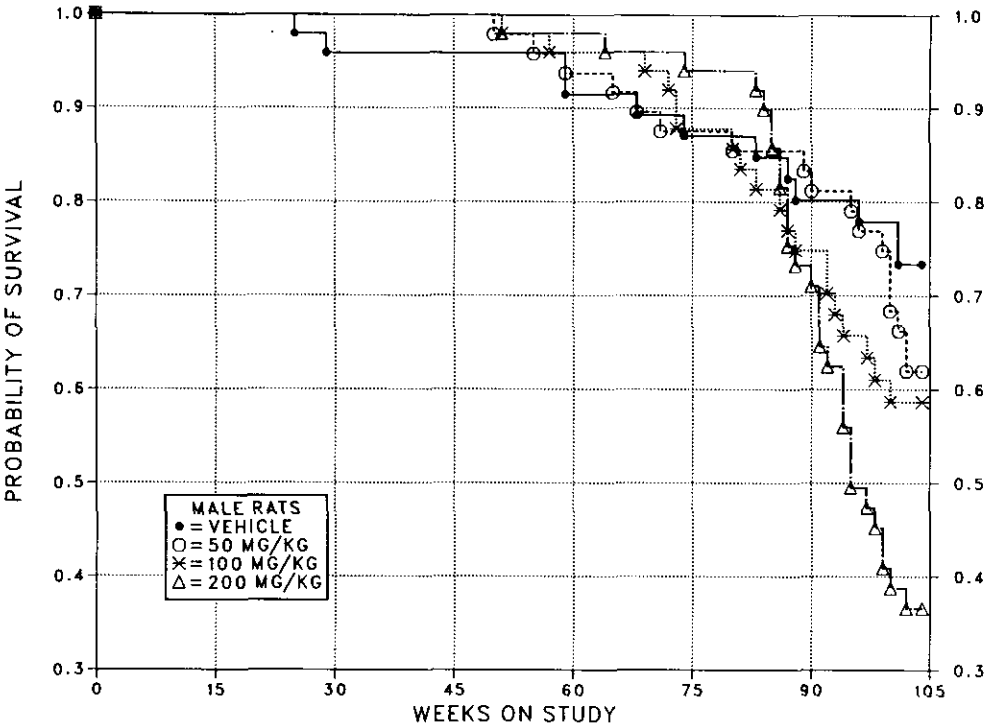

FIGURE 5. Kaplan-Meier survival curves for male rats administered benzene by gavage.

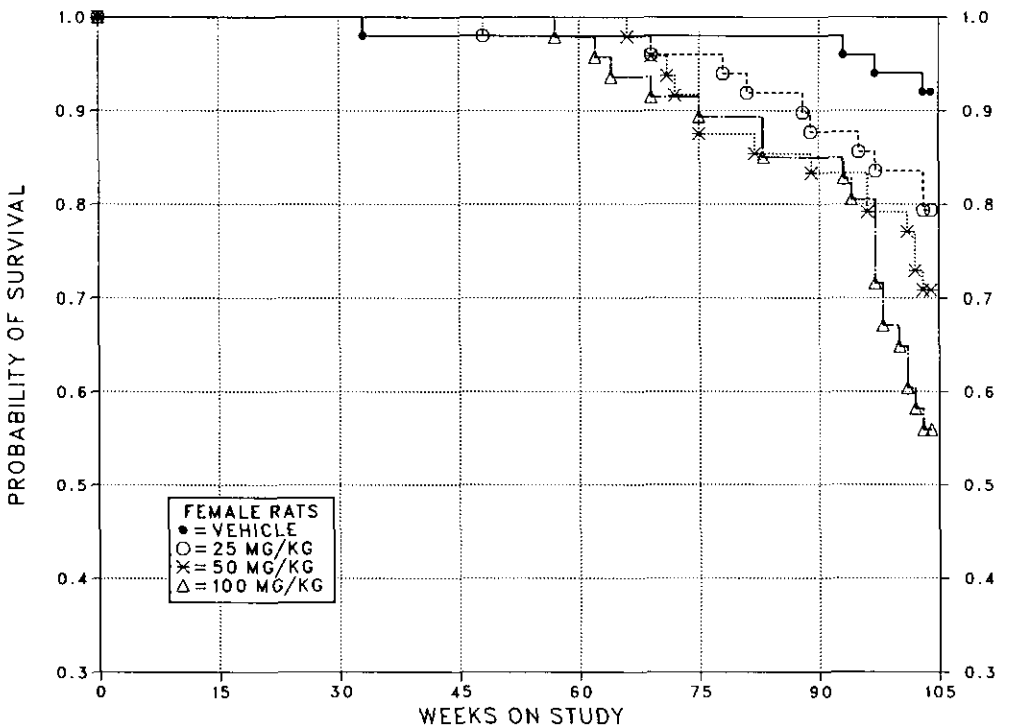

FIGURE 6. Kaplan-Meier survival curves for female rats administered by benzene by gavage.

Table 4. Survival of rats in the 2-year gavage studies of benzene.

Animal disposition	Vehicle control	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg
Male					
Animals initially in study	60		60	60	60
Animals removed for evaluation at 12 months	10		10	10	10
Natural deaths	4		10	7	11
Moribund kills	8		8	13	19
Accidentally killed[a]	6		3	6	4
Animals surviving until end of study (104 weeks)	32		29	24	16
Survival p values[d]	0.001		0.431	0.253	0.003
Female					
Animals initially in study	60	60	60	60	
Animals removed for evaluation at 12 months	10	10	10	10	
Natural deaths	1	2	5	4	
Moribund kills	3	8	10	16	
Accidentally killed[a]	0	2	2	5	
Animals surviving until end of study (104 weeks)	46	38	33	25	
Survival p values[b]	0.001	0.125	0.014	< 0.001	

[a]Exact cause of death not known.
[b]The result of the life table trend test is in the vehicle control column, and the results of the life table pairwise comparisons with the vehicle controls are in the dosed columns.

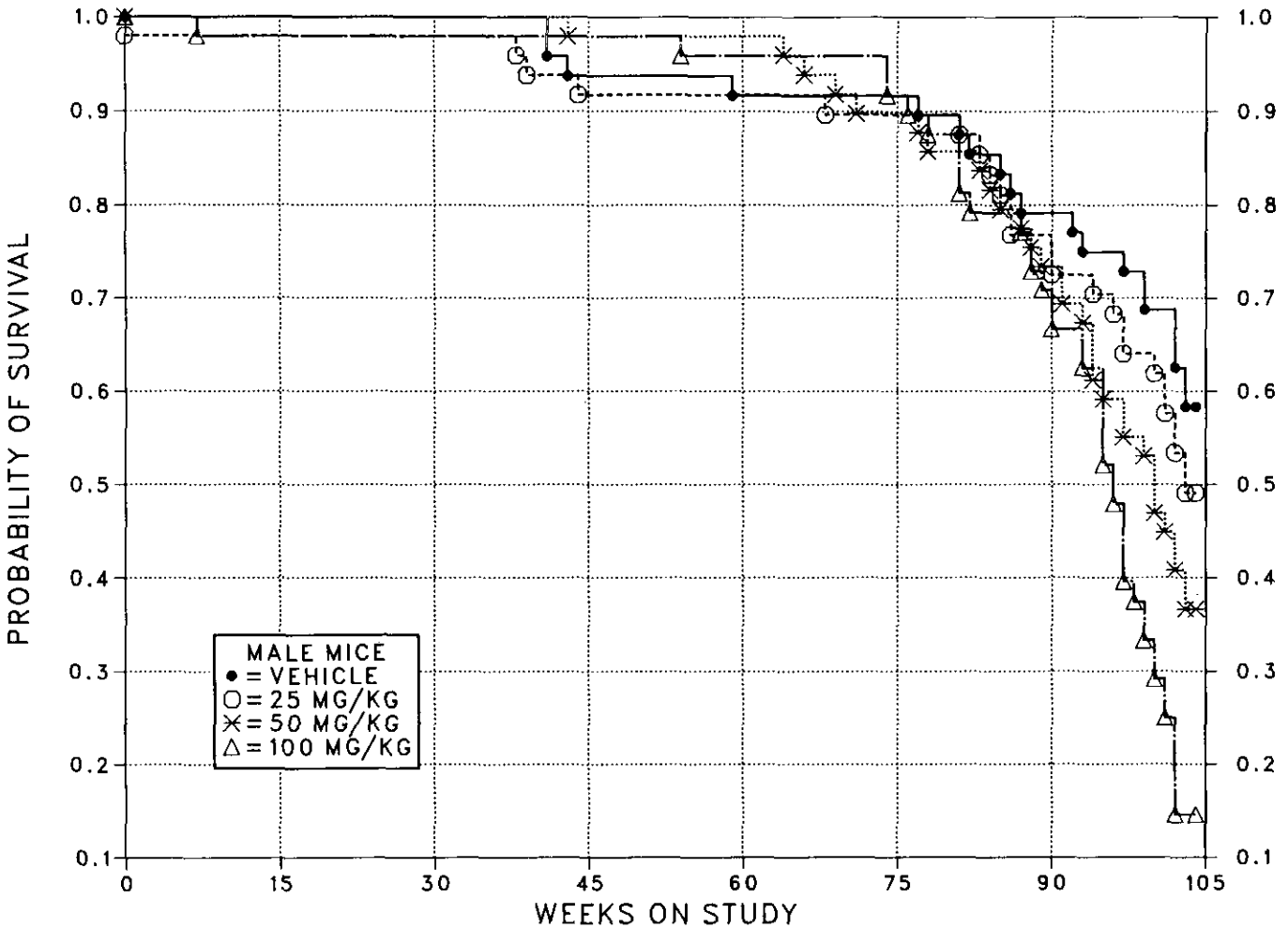

FIGURE 7. Kaplan-Meier survival curves for male mice administered benzene by gavage.

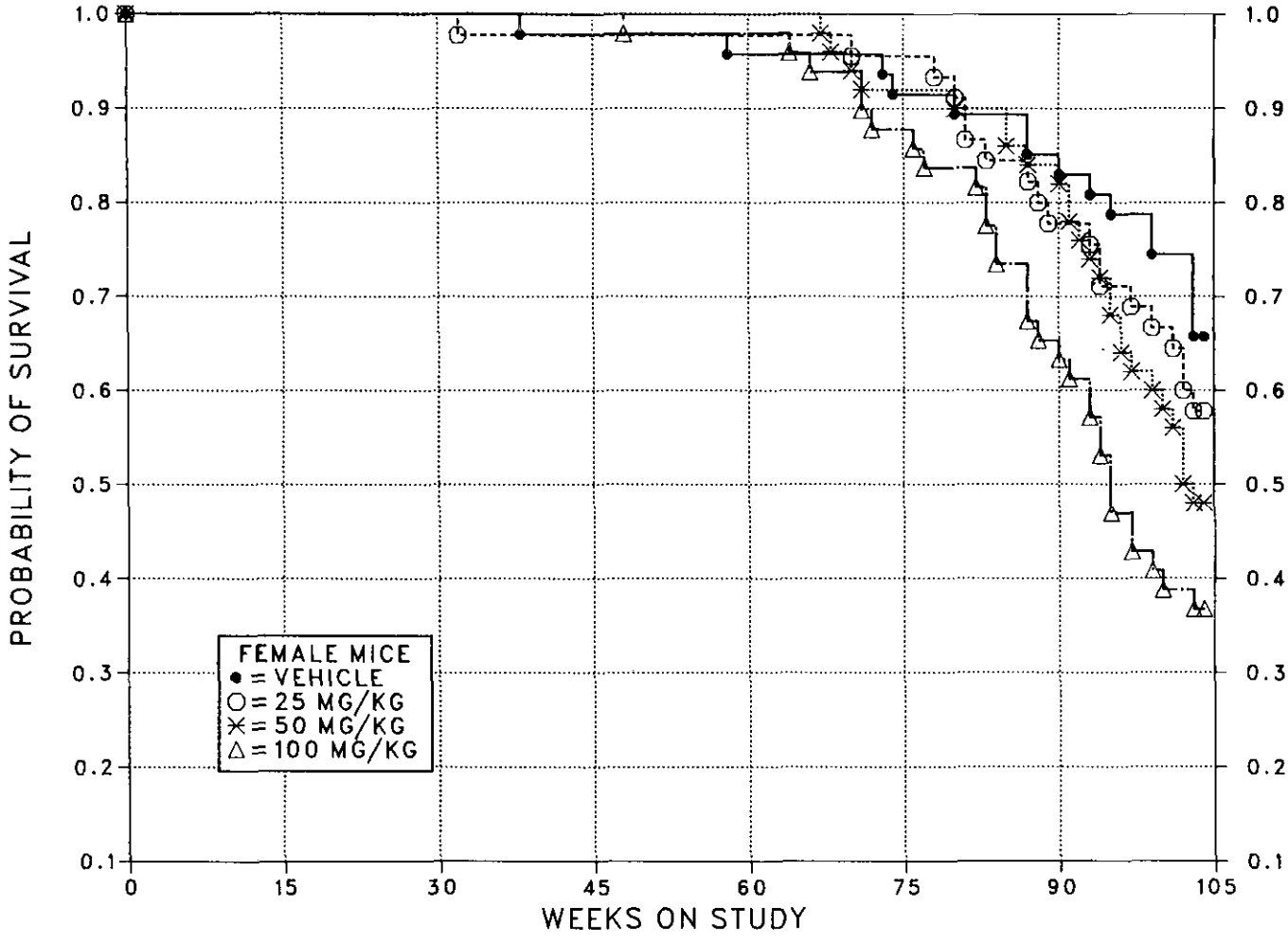

FIGURE 8. Kaplan—Meier survival curves for female mice administered benzene by gavage.

Table 5. Survival of mice in the 2-year gavage studies of benzene.

Animal disposition	Vehicle control	25 mg/kg	50 mg/kg	100 mg/kg
Male				
Animals initially in study	60	60	60	60
Animals removed for evaluation at 12 months	10	10	10	10
Natural deaths	14	18	15	20
Moribund kills	6	7	16	21
Killed accidentally[a]	2	3	1	2
Animals surviving until end of study (104 weeks)	28	22	18	7
Survival p values[b]	< 0.001	0.450	0.059	< 0.001
Female				
Animals initially in study	60	60	60	60
Animals removed at 1 year	10	10	10	10
Natural deaths	10	12	9	17
Moribund kills	6	8	17	16
Killed accidentally[a]	4	5	0	1
Animals surviving until end of study (104 weeks)	30	25	24	16
Survival p values[b]	0.001	0.499	0.110	0.004

[a]Exact cause of death not known.

[b]The result of the life table trend test is in the vehicle control column, and the results of the life table pairwise comparisons with the vehicle controls are in the dosed columns.

Pathology and Statistical Analyses of Results. This section describes the statistically significant or biologically noteworthy changes in the incidence of rats and mice with neoplastic or nonneoplastic lesions. For rats these include Zymbal gland, palate, lip, tongue, skin, uterus, uterus/endometrium, spleen, thymus, cardiac stomach, adrenal gland, thyroid gland, and pituitary gland. For mice these include Zymbal gland, preputial gland, ovary, mammary gland, Harderian gland, lung, hematopoietic system, stomach, liver, and adrenal gland.

RATS. *Zymbal Gland.* Hyperplasia or squamous metaplasia of the Zymbal gland was increased in low-dose male rats and in mid-dose and high-dose females (Table 6). The incidences of ductal dilatation in males (vehicle control, 8/32; low dose, 35/46; mid dose, 20/42; high dose, 28/42) and in females (15/45; 22/40; 28/44; 23/46) were increased as were those of cystic ducts (male: vehicle control, 1/32; low dose, 7/46; mid dose, 6/42; high dose, 0/42; female: 1/45; 2/40; 8/44; 3/46). Carcinomas in males and females occurred with positive trends, and the incidences in the mid-dose and high-dose males and dosed females were greater than those in the vehicle controls (Table 6).

Grossly, these lesions appeared on the side of the head adjacent to or involving the ear and were up to 6 cm in diameter. The interior of the tumor was soft, yellow to white, and occasionally gritty. Microscopically, the tumors contained various amounts of epithelial and sebaceous elements and were classified as adenomas or carcinomas. Rarely, Zymbal gland neoplasms contained significant spindle-cell components; these were classified as carcinosarcomas, and one was found in a 50 mg/kg female rat. Although they vary histologically, these tumors are considered to be part of the spectrum of Zymbal gland tumors. No lesions were observed in the 10 animals per sex killed at 12 months.

Palate, Lip, and Tongue. The numbers of rats with squamous cell neoplasms of the palate, lip, or tongue are given in Table 7. Squamous cell papillomas, squamous cell carcinomas, and squamous cell papillomas or carcinomas (combined) of the palate, lip, or tongue (separately and combined in males and combined in females) occurred with positive trends (Table 8).

Neoplasms of the tongue appeared grossly as raised papillary masses on the dorsal surface. These lesions were well differentiated and contained primarily squamous cells and were classified as either squamous cell papillomas or squamous cell carcinomas depending on whether invasion of adjacent structures had occurred.

Lesions of the lip had a similar microscopic appearance to those neoplasms of the skin and others at the mucocutaneous junction. Although separated topographically, these can be combined with skin tumors for biologic interpretation.

Skin. Squamous cell papillomas, squamous cell carcinomas, and squamous cell papillomas or carcinomas (combined) in male rats occurred with positive trends, and the incidences in the high-dose group were greater than those in the vehicle controls (Table 9).

Neoplasms of the skin were found on the face, back, flank, and other locations. The lesions varied from 1 to 3 cm in diameter and were raised, ulcerated, and crusty, often with a yellow, friable center. Microscopically, the lesions varied from squamous cell papillomas and squamous cell carcinomas to neoplasms containing various amounts of adnexal structures. If sebaceous, basal, and squamous elements were found, these lesions were classified as adenosquamous adenomas and carcinomas. A few tumors formed primarily hair follicles (classified as trichoepitheliomas), sebaceous elements (classified as sebaceous adenomas), or craterlike epithelial tumors (clas-

Table 6. Analysis of Zymbal gland lesions in rats in the 2-year gavage studies of benzene.[a]

Zymbal gland lesions	Vehicle control	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg
Male					
Hyperplasia	0/32 (0%)[b]		6/46 (13%)	2/42 (3%)	3/42 (7%)
Adenoma	0/32 (0%)		1/46 (2%)	0/42 (0%)	1/42 (2%)
Carcinoma	2/32 (6%)		6/46 (13%)	10/42 (24%)	17/42 (40%)
Life table tests	$p < 0.001$		$p = 0.193$	$p = 0.017$	$p < 0.001$
Incidental tumor tests	$p = 0.003$		$p = 0.352$	$p = 0.214$	$p = 0.024$
Adenoma or carcinoma[c]	2/32 (6%)		7/46 (15%)	10/42 (24%)	18/42 (43%)
Life table tests	$p < 0.001$		$p = 0.131$	$p = 0.017$	$p < 0.001$
Incidental tumor tests	$p = 0.002$		$p = 0.247$	$p = 0.214$	$p = 0.019$
Female					
Hyperplasia	0/45 (0%)	0/40 (0%)	6/44 (14%)	1/46 (2%)[b]	
Adenoma	0/45 (0%)	0/40 (0%)	1/44 (2%)	1/46 (2%)	
Carcinoma	0/45 (0%)	5/40 (10%)	5/44 (11%)	14/46 (30%)	
Life table tests	$p < 0.001$	$p = 0.022$	$p = 0.018$	$p < 0.001$	
Incidental tumor tests	$p < 0.001$	$p = 0.036$	$p = 0.067$	$p < 0.001$	
Adenoma or carcinoma[d]	0/45 (0%)	5/40 (13%)	6/44 (14%)[e]	15/46 (33%)	
Life table tests	$p < 0.001$	$p = 0.022$	$p = 0.010$	$p < 0.001$	
Incidental tumor tests	$p < 0.001$	$p = 0.036$	$p = 0.021$	$p < 0.001$	

[a]The statistical analyses used are given by Haseman (*180*).
[b]One vehicle control male rat and two high-dose female rats had squamous metaplasia.
[c]Historical incidence at laboratory (mean): 0/100; historical incidence in NTP studies: 4/1146 (0.3%).
[d]Historical incidences at laboratory (mean): 1/100 (1%); historical incidence in NTP studies: 5/1147 (0.4%).
[e]A carcinosarcoma was observed in a seventh mid-dose female rat, and a squamous cell papilloma of the ear was observed in an eighth mid-dose female rat.

Table 7. Number of rats with squamous cell neoplasms of the palate, lip, and tongue in the 2-year gavage studies of benzene.[a]

	Male				Female			
Oral cavity lesions	0	50 mg/kg	100 mg/kg	200 mg/kg	0	25 mg/kg	50 mg/kg	100 mg/kg
No. of rats examined	50	50	50	50	50	50	50	50
Palate								
Papilloma	0	4	4	9	1	3	5	3
Carcinoma	0	0	1	0	0	1	0	1
Papilloma or carcinoma	0	4	5	9	1	4	5	4
Tongue								
Papilloma	1	0	2	2	0	1	1	0
Carcinoma	0	3	4	4	0	0	4	4
Papilloma or carcinoma	1	3	6	6	0	1	5	4
Lip								
Papilloma	0	2	5	5	0	0	2	2
Carcinoma	0	0	0	3	0	0	0	0
Papilloma or carcinoma	0	2	5	8	0	0	2	2
Palate, tongue, or lip								
Papilloma	1	6	11	13	1	4	8	5
Carcinoma	0	3	5	7	0	1	4	5
Papilloma or carcinoma	1	9	16	19	1	5	12	9

[a]Results of statistical analyses are shown in Table 8.

Table 8. Analysis of palate, lip, and tongue tumors in rats in the 2-year gavage studies of benzene.

Oral cavity lesions	Vehicle control	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg
Male					
Palate: squamous cell papilloma or carcinoma	0/50 (0%)		4/50 (8%)	5/50 (10%)	9/50 (18%)
Tongue: squamous cell papilloma or carcinoma	1/50 (2%)		3/50 (6%)	6/50 (12%)	6/50 (12%)
Lip: squamous cell papilloma or carcinoma	0/50 (0%)		2/50 (4%)	5/50 (10%)	8/50 (16%)
Life table tests	$p < 0.001$		$p = 0.058$	$p = 0.001$	$p = 0.001$
Incidental tumor tests	$p < 0.001$		$p = 0.216$	$p = 0.015$	$p = 0.002$
All oral cavity: squamous cell papilloma	1/50 (2%)		6/50 (12%)	11/50 (22%)	13/50 (26%)
Life table tests	$p < 0.001$		$p = 0.058$	$p = 0.001$	$p < 0.001$
Incidental tumor tests	$p < 0.001$		$p = 0.083$	$p = 0.004$	$p = 0.002$
All oral cavity: squamous cell carcinoma	0/50 (0%)		3/50 (6%)	5/50 (10%)	7/50 (14%)
Life table tests	$p < 0.001$		$p = 0.133$	$p = 0.030$	$p = 0.001$
Incidental tumor tests	$p = 0.002$		$p = 0.133$	$p = 0.071$	$p = 0.001$
All oral cavity: squamous cell papilloma or carcinoma[a]	1/50 (2%)		9/50 (18%)	16/50 (32%)	19/50 (38%)
Life table tests	$p < 0.001$		$p = 0.012$	$p < 0.001$	$p < 0.001$
Incidental tumor tests	$p < 0.001$		$p = 0.014$	$p < 0.001$	$p < 0.001$
Female					
Palate: squamous cell papilloma or carcinoma	1/50 (2%)	4/50 (8%)	5/50 (10%)	4/50 (8%)	
Tongue: squamous cell papilloma or carcinoma	0/50 (0%)	1/50 (2%)	5/50 (10%)	4/50 (8%)	
Lip: squamous cell papilloma	0/50 (0%)	0/50 (0%)	2/50 (4%)	2/50 (4%)	
All oral cavity: squamous cell papilloma	1/50 (2%)	4/50 (8%)	8/50 (16%)	5/50 (10%)	
Life table tests	$p = 0.017$	$p = 0.127$	$p = 0.006$	$p = 0.032$	
Incidental tumor tests	$p = 0.047$	$p = 0.127$	$p = 0.022$	$p = 0.121$	
All oral cavity: squamous cell carcinoma	0/50 (0%)	1/50 (2%)	4/50 (8%)	5/50 (10%)	
Life table tests	$p = 0.003$	$p = 0.468$	$p = 0.047$	$p = 0.010$	
Incidental tumor tests	$p = 0.201$	$p = 0.557$	$p = 0.240$	$p = 0.184$	
Oral cavity: squamous cell papilloma or carcinoma[b]	1/50 (2%)	5/50 (10%)	12/50 (24%)	9/50 (18%)	
Life table tests	$p < 0.001$	$p < 0.068$	$p = 0.001$	$p = 0.001$	
Incidental tumor tests	$p = 0.039$	$p = 0.081$	$p = 0.007$	$p = 0.029$	

[a]Historical incidence of oral cavity tumors (palate, tongue, or oral cavity) at laboratory (mean): 0/100; historical incidence in NTP studies: 2/1,146 (0.2%).
[b]Historical incidence of oral cavity tumors (palate, tongue, or oral cavity) at laboratory (mean): 1/100 (1%); historical incidence in NTP studies: 3/1147 (0.3%).

sified as keratoacanthomas). These tumors represent a spectrum arising from the skin and adnexal tissues and can be combined.

Uterus. Incidences of epithelial hyperplasia in dosed and vehicle control female rats were comparable (Table 10). Endometrial stromal polyps occurred with a positive trend in female rats, and the incidence in the high-dose group was greater than that in the vehicle controls.

Uterus/Endometrium. Endometrial carcinomas were found in two low-dose rats, adenomas were found in one low-dose rat, and adenocarcinomas were found in two mid-dose and two high-dose rats; one adenosquamous carcinoma of the cervix was found in a mid-dose rat.

Spleen. Lymphoid depletion in the spleen was observed at increased incidences in dosed male and female rats (male: vehicle control, 0/49; low dose, 19/48; 40%; mid

Table 9. Analysis of skin tumors in male rats in the 2-year gavage studies of benzene.

Skin lesions	Vehicle control	50 mg/kg	100 mg/kg	200 mg/kg
Squamous cell papilloma	0/50 (0%)	2/50 (4%)	1/50 (2%)	5/50 (10%)
Life table tests	$p = 0.001$	$p = 0.216$	$p = 0.451$	$p = 0.005$
Incidental tumor tests	$p = 0.002$	$p = 0.216$	$p = 0.451$	$p = 0.009$
Squamous cell carcinoma	0/50 (0%)	5/50 (10%)	3/50 (6%)	8/50 (16%)
Life table tests	$p < 0.001$	$p = 0.032$	$p = 0.098$	$p = 0.001$
Incidental tumor tests	$p = 0.069$	$p = 0.064$	$p = 0.278$	$p = 0.039$
All squamous cell neoplasms	1/50 (2%)	7/50 (14%)	5/50 (10%)	12/50 (24%)
Life table tests	$p < 0.001$	$p = 0.031$	$p = 0.076$	$p < 0.001$
Incidental tumor tests	$p = 0.003$	$p = 0.055$	$p = 0.221$	$p < 0.001$
Squamous cell tumors or undifferentiated carcinoma[a]	1/50 (2%)	7/50 (14%)	5/50 (10%)	13/50 (26%)
Life table tests	$p < 0.001$	$p = 0.031$	$p = 0.076$	$p < 0.001$
Incidental tumor tests	$p = 0.002$	$p = 0.055$	$p = 0.221$	$p < 0.001$

[a]Historical incidence at laboratory (mean ± SD): 1/100 (1%); historical incidence in NTP studies: 2/1146 (2% ± 3%).

Table 10. Analysis of uterine lesions in female rats in the 2-year gavage studies of benzene.

Uterine lesions	Vehicle control	25 mg/kg	50 mg/kg	100 mg/kg
Hyperplasia (focal, diffuse, papillary, or cystic)	33/50 (66%)	35/50 (70%)	34/49 (69%)	35/50 (70%)
Endometrial stromal polyp[a]	7/50 (14%)	7/50 (14%)	7/49 (14%)	14/50 (28%)
Life table tests	$p < 0.001$	$p = 0.468$	$p = 0.420$	$p = 0.003$
Incidental tumor tests	$p = 0.049$	$p = 0.545$	$p = 0.598$	$p = 0.049$

[a]Historical incidence at laboratory (mean ± SD): 22/98 (22%); historical incidence in NTP studies: 248/1125 (22% ± 7%).

dose, 8/47, 17%; high dose, 23/47, 49%; female: vehicle control, 0/50; low dose, 11/50, 22%; mid dose, 8/49, 16%; high dose, 10/49, 20%).

Thymus. Lymphoid depletion was observed at increased incidence in dosed male rats (vehicle control, 0/44; low dose, 4/42, 10%; mid dose, 8/41, 20%; high dose, 10/34, 29%).

Cardiac (Nonglandular) Stomach. Hyperkeratosis and acanthosis in the nonglandular forestomach were observed at increased incidences in high-dose male rats (hyperkeratosis: vehicle control, 2/48, 4%; low dose, 4/44, 9%; mid dose 3/48, 6%; high dose, 9/47, 19%; acanthosis: vehicle control, 2/48, 4%; low dose, 4/44, 9%; mid dose, 3/48, 6%; high dose, 10/47, 21%).

Adrenal Gland—Zona Fasciculata. Hyperplasia was observed at increased incidences in low-dose rats of each sex (male: vehicle control, 0/50; low dose, 13/49, 27%; mid dose, 0/48; high dose, 2/49, 4%; female: vehicle control, 0/50, low dose, 17/50, 34%; mid dose, 0/47; high dose, 0/49).

Thyroid Gland. C-cell adenomas or carcinomas (combined) in male rats occurred with a negative trend ($p = 0.039$) and the incidences in the dosed groups were not significantly different from that in the vehicle control group (vehicle control, 9/49, 18%; low dose, 5/47, 11%; mid dose, 4/46, 9%; high dose, 2/47, 4%). The incidences of C-cell hyperplasia were 7/49 (14%) in the vehicle control, 12/47 (26%) in the low-dose, 7/46 (15%) in the mid-dose, and 7/47 (15%) in the high-dose groups.

Pituitary Gland. Adenomas in male and female rats and carcinomas in male rats occurred with negative trends. The incidences in high-dose females and in mid-dose and high-dose males were lower than those of the vehicle controls. In male rats, the incidence of hyperplasia was as follows: vehicle control, 3/47 (6%); low dose, 7/45 (16%); mid dose, 9/46 (20%); high dose, 5/48 (10%); the incidence of adenomas or carcinomas (combined) was 18/47 (38%) in the vehicle control, 11/45 (24%) in the low-dose, 11/46 (24%) in the mid-dose, and 7/48 (15%) in the high-dose groups. In female rats, the incidence of hyperplasia was as follows: vehicle control, 5/47 (11%); low dose, 10/50 (20%); mid dose, 5/48 (10%); high dose, 7/49 (14%); the incidence of adenomas was 22/47 (47%) in the vehicle control, 15/50 (30%) in the low-dose, 15/48 (31%) in the mid-dose, and 8/49 (16%) in the high dose groups.

MICE. *Zymbal Gland.* Epithelial hyperplasia was observed at increased incidences in mid- and high-dose male and high-dose female mice (Table 11). Squamous cell carcinomas in males and females occurred with positive trends. The incidences in the mid- and high-dose males and high-dose females were greater than those in the vehicle controls. All neoplasms were carcinomas; no adenomas were observed. Also, one high-dose male and one high-dose female had a carcinosarcoma.

The incidences of Zymbal gland carcinomas were increased in mid-dose and high-dose male mice and in high-dose female mice. In mid-dose and high-dose male mice and in high-dose female mice, the incidences of epithelial hyperplasia of the Zymbal gland were also increased.

The possible early occurrence and progression of these lesions was investigated by an examination of the Zymbal glands from the 10 animals per group killed after 12 months of exposure; no nonneoplastic or neoplastic pathologic effects were observed.

Preputial Gland. Hyperplasia was observed at increased incidences in the preputial gland of low and mid-dose male mice (Table 12). Squamous cell carcinomas and

Table 11. Analysis of Zymbal gland lesions in mice in the 2-year gavage studies of benzene.

Zymbal gland lesions	Vehicle control	25 mg/kg	50 mg/kg	100 mg/kg
Male				
Epithelial hyperplasia	0/43 (0%)	3/34 (9%)	12/40 (30%)	10/39 (26%)
Squamous cell carcinoma[a]	0/43 (0%)	1/34 (3%)	4/40 (10%)	21/39 (54%)
Life table tests	$p < 0.001$	$p = 0.489$	$p = 0.012$	$p < 0.001$
Incidental tumor tests	$p < 0.001$	$p = 0.500$	$p = 0.012$	$p < 0.001$
Female				
Epithelial hyperplasia	1/43 (2%)	1/32 (3%)	2/37 (5%)	6/31 (19%)
Squamous cell carcinoma[b]	0/43 (0%)	0/32 (0%)	1/37 (3%)	3/31 (10%)
Life table tests	$p = 0.007$	—[c]	$p = 0.450$	$p = 0.045$
Incidental tumor tests	$p = 0.007$	—[c]	$p = 0.450$	$p = 0.045$

[a]Historical incidence at laboratory (mean): 0/100; historical incidence in NTP studies: 0/1090.
[b]Historical incidence at laboratory (mean): 0.99; historical incidence in NTP studies: 1/1187 (< 0.1%).
[c]No p values are reported because no tumors were observed in the 25 mg/kg and vehicle control groups.

Table 12. Analysis of preputial gland lesions in male mice in the 2-year gavage studies of benzene.

Preputial gland lesions	Vehicle control	25 mg/kg	50 mg/kg	100 mg/kg
Hyperplasia (focal, diffuse, or epithelial)	1/21 (5%)	18/28 (65%)	9/29 (31%)	1/35 (3%)
Squamous cell carcinoma	0/21 (0%)	3/28 (11%)	18/29 (62%)	28/35 (80%)
Life table tests	$p < 0.001$	$p = 0.225$	$p < 0.001$	$p < 0.001$
Incidental tumor tests	$p < 0.001$	$p = 0.225$	$p < 0.001$	$p < 0.001$
Carcinoma, NOS	0/21 (0%)	2/28 (7%)	1/29 (3%)	3/35 (9%)
Life table tests	$p = 0.019$	$p = 0.359$	$p = 0.445$	$p = 0.043$
Incidental tumor tests	$p = 0.234$	$p = 0.359$	$p = 0.545$	$p = 0.234$
Carcinoma (all types)[a]	0/21 (0%)	5/28 (18%)	19/29 (66%)	31/35 (89%)
Life table tests	$p < 0.001$	$p = 0.091$	$p < 0.001$	$p < 0.001$
Incidental tumor tests	$p < 0.001$	$p = 0.091$	$p < 0.001$	$p < 0.001$

[a]Historical incidence of adenomas or carcinomas at laboratory (mean): 0/100; historical incidence in NTP studies: 1/1090 (< 0.1%).

Table 13. Number of female mice with ovarian lesions in the 2-year gavage studies of benzene.[a]

Ovarian lesions	Vehicle control	25 mg/kg	50 mg/kg	100 mg/kg
No. of mice examined	47	44	49	48
Epithelial hyperplasia	12	39	31	29
Senile atrophy	15	35	32	22
Papillary cystadenoma	0	0	2	1
Luteoma	0	2	3	2
Tubular adenoma	0	0	3	3
Granulosa cell tumor	1	1	6	7
Granulosa cell carcinoma	0	0	0	1
Benign mixed tumor	0	1	12	7

[a]Results of statistical analyses are shown in Table 14.

carcinomas (all types) occurred in male mice with positive trends. The incidences of squamous cell carcinomas in mid- and high-dose males and the incidences of carcinomas (all types) in mid- and high-dose males were greater than those of the vehicle controls. One low-dose and one high-dose male had a carcinosarcoma. There were no corresponding clitoral gland neoplasms in females. Epithelial hyperplasia was observed in six low-dose, two mid-dose, and one high-dose female.

Ovary. The incidence of female mice with various nonneoplastic and neoplastic lesions in the ovary is given in Table 13. Granulosa cell tumors and benign mixed tumors occurred with positive trends. The incidences of granulosa cell tumors in the high-dose group and benign mixed tumors in the mid- and high-dose groups were greater than those in the vehicle controls (Table 14). The granulosa cell neoplasms consisted of well differentiated granulosa cells arranged in tubular patterns, cell clusters separated by interconnecting strands of stromal connective tissue, or homogeneous cell populations with no definite cellular arrangement and scanty stroma. Benign mixed tumors comprised heterogeneous cell types including tubular structures arising from the ovarian surface epithelium (germinal epithelium), stromal cells, and/or granulosa cells exhibiting varying degrees of luteinization. Luteomas consisted predominantly of luteinized theca and/or granulosa cells and tubular adenomas consisted of randomly arranged tubules with a low cuboidal epithelium.

Mammary Gland. Carcinomas and carcinosarcomas in female mice occurred with positive trends (Table 15). The incidences of carcinomas in mid- and high-dose female mice and of carcinosarcomas in high-dose female mice were greater than those in the vehicle controls. Carci-

Table 14. Analysis of ovarian tumors in female mice in the 2-year gavage studies of benzene.[a]

Ovarian lesions	Vehicle control	25 mg/kg	50 mg/kg	100 mg/kg
Tubular adenoma	0/47 (0%)	0/44 (0%)	3/49 (6%)	3/48 (6%)
Life table tests	$p = 0.008$	—[b]	$p = 0.090$	$p = 0.047$
Incidental tumor tests	$p = 0.016$	—[b]	$p = 0.119$	$p = 0.077$
Granulosa cell tumor	1/47 (2%)	1/44 (2%)	6/49 (12%)	7/48 (15%)
Life table tests	$p < 0.001$	$p = 0.730$	$p = 0.040$	$p = 0.008$
Incidental tumor tests	$p = 0.005$	$p = 0.730$	$p = 0.077$	$p = 0.020$
Granulosa cell tumor or carcinoma[c]	1/47 (2%)	1/44 (2%)	6/49 (12%)	8/48 (17%)
Life table tests	$p < 0.001$	$p = 0.730$	$p = 0.040$	$p = 0.004$
Incidental tumor tests	$p = 0.002$	$p = 0.730$	$p = 0.077$	$p = 0.012$
Benign mixed tumor	0/47 (0%)	1/44 (2%)	12/49 (24%)	7/48 (15%)
Life table tests	$p < 0.001$	$p = 0.471$	$p < 0.001$	$p = 0.001$
Incidental tumor tests	$p < 0.001$	$p = 0.471$	$p < 0.001$	$p = 0.002$

[a]Historical incidence of ovarian tumors at laboratory: 0/100; historical incidence in NTP studies: 9/1028 (0.09%); no more than two ovarian tumors were present in any single control group.
[b]No p values are reported because no tumors were observed in the 25 mg/kg and vehicle control groups.
[c]Historical incidence in NTP studies (mean): 3/1028 (0.3%).

Table 15. Analysis of mammary gland lesions in female mice in the 2-year gavage studies of benzene.

Mammary gland lesions	Vehicle control	25 mg/kg	50 mg/kg	100 mg/kg
Hyperplasia (focal or cystic)	2/49 (4%)	4/45 (9%)	2/50 (4%)	1/49 (2%)
Carcinoma[a]	0/49 (0%)	2/45 (4%)	5/50 (10%)	10/49 (20%)
Life table tests	$p < 0.001$	$p = 0.202$	$p = 0.026$	$p < 0.001$
Incidental tumor tests	$p < 0.001$	$p = 0.233$	$p = 0.047$	$p = 0.004$
Carcinosarcoma	0/49 (0%)	0/45 (0%)	1/50 (2%)	4/49 (8%)
Life table tests	$p = 0.001$	—[b]	$p = 0.495$	$p = 0.017$
Incidental tumor tests	$p = 0.003$	—[b]	$p = 0.588$	$p = 0.030$

[a]Historical incidence of carcinomas at laboratory (mean ± SD): 1/99 (1%); historical incidence in NTP studies: 15/1187 (1% ± 2%).
[b]No p values are reported because no tumors were observed in the 25 mg/kg and vehicle control groups.

Table 16. Analysis of Harderian gland lesions in mice in the 2-year gavage studies of benzene.

Harderian gland lesions	Vehicle control	25 mg/kg	50 mg/kg	100 mg/kg
Male				
Focal hyperplasia	0/49 (0%)	5/46 (11%)	11/49 (22%)	7/48 (15%)
Adenoma[a]	0/49 (0%)	9/46 (20%)	13/49 (27%)	11/48 (23%)
Life table tests	$p < 0.001$	$p = 0.001$	$p < 0.001$	$p < 0.001$
Incidental tumor tests	$p = 0.001$	$p = 0.001$	$p < 0.001$	$p = 0.003$
Carcinoma	1/49 (2%)	2/46 (4%)	0/49 (0%)	3/48 (6%)
Life table tests	$p = 0.064$	$p = 0.423$	$p = 0.588$N[b]	$p = 0.128$
Incidental tumor tests	$p = 0.309$	$p = 0.467$	$p = 0.588$N	$p = 0.408$
Adenoma or carcinoma	1/49 (2%)	10/46 (22%)	13/49 (27%)	14/48 (29%)
Life table tests	$p < 0.001$	$p = 0.002$	$p < 0.001$	$p < 0.001$
Incidental tumor tests	$p < 0.001$	$p = 0.003$	$p < 0.001$	$p = 0.002$
Female				
Focal or diffuse hyperplasia	6/48 (13%)	10/44 (23%)	11/50 (22%)	10/47 (21%)
Adenoma[c]	5/48 (10%)	6/44 (14%)	10/50 (20%)	6/47 (13%)
Life table tests	$p = 0.133$	$p = 0.369$	$p < 0.090$	$p = 0.204$
Incidental tumor tests	$p = 0.311$	$p = 0.369$	$p < 0.209$	$p = 0.281$
Carcinoma	0/48 (0%)	0/44 (0%)	0/50 (0%)	4/47 (9%)
Life table tests	$p < 0.001$	—[d]	—[d]	$p = 0.020$
Incidental tumor tests	$p = 0.003$	—[d]	—[d]	$p = 0.060$
Adenoma or carcinoma	5/48 (10%)	6/44 (14%)	10/50 (20%)	10/47 (21%)
Life table tests	$p = 0.009$	$p = 0.369$	$p = 0.090$	$p = 0.017$
Incidental tumor test	$p = 0.046$	$p = 0.369$	$p = 0.209$	$p = 0.049$

[a]Historical incidence at laboratory (mean ± SD); 1/100 (1%); historical incidence in NTP studies: 32/1090 (3% ± 3%). Two carcinomas were observed.
[b]N = decrease compared to controls.
[c]Historical incidence at laboratory (mean ± SD): 0/99; historical incidence in NTP studies: 11/1187 (0.9% ± 1%). One carcinoma was observed.
[d]No p values are reported because no tumors were observed in the 25 mg/kg or 50 mg/kg and vehicle control groups.

nomas often showed both an organoid pattern of epithelial cell arrangement and extensive squamous differentiation. Carcinosarcomas consisted of highly anaplastic epithelial cells and a prominent spindle-cell component resembling malignant fibroblasts.

Harderian Gland. Focal hyperplasia was observed at increased incidences in dosed male and female mice (Table 16). Adenomas in males and carcinomas in females occurred with positive trends. The incidences of adenomas in dosed male mice were greater than that in the vehicle controls. The incidence of carcinomas in high-dose females was marginally greater than that in vehicle controls. These neoplasms consisted of pseudoglandular structures lined by epithelium showing loss of polarity, mild to moderate pleomorphism, and formation of multiple layers. The malignant tumors were less differentiated and were locally invasive.

Harderian glands were examined from the 10 animals per group killed at 12 months. Mild localized epithelial hyperplasia was observed in 1/10 female vehicle controls, 1/10 low-dose males, and 1/10 mid-dose females; this latter mouse had a small adenoma near the hyperplastic focus. One of 10 low-dose females had a small focus of necrosis and acute inflammation.

Lung. The incidences of alveolar epithelial hyperplasia and alveolar/bronchiolar neoplasms were increased in dosed male and female mice (Table 17). Most of the significant increases were due to carcinomas. For male mice, the life table and incidental tumor tests give different results regarding the statistical significance of dose-response trends and of increased tumor incidences in the mid- and high-dose groups. In this instance, the life table test should be given the greater emphasis, since (a) the increased incidence of lung tumors is due primarily to malignant, potentially life-threatening tumors, (b) the incidental tumor test has somewhat reduced sensitivity because of the excessive mortality in the high-dose group, and (c) the unadjusted analyses indicate a significant increase in tumor incidence, despite the reduced survival in the high-dose group.

Hematopoietic System. Hematopoietic hyperplasia was observed at increased incidences in the bone marrow of dosed mice of each sex (Table 18). Splenic hematopoiesis was also increased with dose (male: vehicle control, 5/49; low dose, 9/48; mid dose; 19/49, high dose, 24/47; female: 9/49; 10/45; 6/50; 14/49). The few neoplasms diagnosed as leukemia (2 in males, 5 in females) were considered to be malignant lymphomas with associated "leukemia" (i.e., evidence of elevated lymphocytes/lymphoblasts in peripheral blood). Thus, the lymphoma or leukemia data (Table 18) should be viewed as all lymphoma for evaluation purposes. Malignant lymphomas in male and female mice occurred with positive trends. The incidences of malignant lymphomas in all dosed groups of males and females were greater than those in the vehicle controls by the life table test, which is generally regarded as the more appropriate method of statistical analysis for these potentially life-threatening neoplasms.

Forestomach. Epithelial hyperplasia and hyperkeratosis were observed at increased incidences in low-dose males and mid-dose and high-dose females (Table 19). Squamous cell papillomas in male mice occurred with a marginal positive trend ($p = 0.048$). The combined incidence of male mice with either squamous cell papillomas or carcinomas, however, did not occur with a positive trend ($p = 0.07$) by the incidental tumor test even though

Table 17. Analysis of lung lesions in mice in the 2-year gavage studies of benzene.

Lung lesions	Vehicle control	25 mg/kg	50 mg/kg	100 mg/kg
Male				
Alveolar epithelial hyperplasia	2/49 (4%)	3/48 (6%)	7/50 (14%)	10/49 (20%)
Alveolar/bronchiolar adenoma	6/49 (12%)	6/48 (13%)	8/50 (16%)	12/49 (24%)
Life table tests	$p < 0.001$	$p = 0.499$	$p = 0.188$	$p = 0.005$
Incidental tumor tests	$p = 0.179$	$p = 0.609$	$p = 0.486$	$p = 0.326$
Alveolar/bronchiolar carcinoma	5/49 (10%)	11/48 (23%)	12/50 (24%)	14/49 (29%)
Life table tests	$p < 0.001$	$p = 0.052$	$p = 0.017$	$p = 0.001$
Incidental tumor tests	$p = 0.046$	$p = 0.083$	$p = 0.083$	$p = 0.073$
Alveolar/bronchiolar adenoma or carcinoma[a]	10/49 (20%)	16/48 (33%)	19/50 (38%)	21/49 (43%)
Life table tests	$p < 0.001$	$p = 0.069$	$p = 0.007$	$p < 0.001$
Incidental tumor tests	$p = 0.056$	$p = 0.124$	$p = 0.070$	$p = 0.094$
Female				
Alveolar epithelial hyperplasia	1/49 (2%)	1/42 (2%)	9/50 (18%)	6/49 (12%)
Alveolar/bronchiolar adenoma	4/49 (8%)	2/42 (5%)	5/50 (10%)	9/49 (18%)
Life table tests	$p = 0.003$	$p = 0.437N$[b]	$p = 0.398$	$p = 0.011$
Incidental tumor tests	$p = 0.010$	$p = 0.435N$	$p = 0.514$	$p = 0.020$
Alveolar/bronchiolar carcinoma	0/49 (0%)	3/42 (7%)	6/50 (12%)	6/49 (12%)
Life table tests	$p = 0.002$	$p = 0.084$	$p = 0.010$	$p = 0.004$
Incidental tumor tests	$p = 0.006$	$p = 0.084$	$p = 0.013$	$p = 0.009$
Alveolar/bronchiolar adenoma or carcinoma[c]	4/49 (8%)	5/42 (12%)	10/50 (20%)	13/49 (27%)
Life table tests	$p < 0.001$	$p = 0.366$	$p = 0.039$	$p < 0.001$
Incidental tumor tests	$p < 0.001$	$p = 0.368$	$p = 0.071$	$p = 0.002$

[a]Historical incidence at laboratory (mean ± SD): 14/100 (14%); historical incidence in NTP studies: 155/1082 (14% ± 6%).
[b]N = decrease compared to controls.
[c]Historical incidence at laboratory (mean ± SD): 4/97 (4%); historical incidence in NTP studies: 52/1103 (5% ± 4%).

Table 18. Analysis of hematopoietic system lesions in mice in the 2-year gavage studies of benzene.

Hematopoietic system lesions	Vehicle control	25 mg/kg	50 mg/kg	100 mg/kg
Male				
Hematopoietic hyperplasia (bone marrow)	0/49 (0%)	11/48 (22%)	10/50 (20%)	25/49 (50%)
Lymphoma, all malignant	4/49 (8%)	9/48 (19%)	9/50 (18%)	15/49 (31%)
Life table tests	$p < 0.001$	$p = 0.075$	$p = 0.030$	$p < 0.001$
Incidental tumor tests	$p = 0.003$	$p = 0.116$	$p = 0.072$	$p = 0.022$
Leukemia	0/49 (0%)	1/48 (21%)	1/50 (2%)	0/49 (0%)
Lymphoma or leukemia[a]	4/49 (8%)	10/48 (21%)	10/50 (20%)	15/49 (31%)
Life table tests	$p < 0.001$	$p = 0.048$	$p = 0.018$	$p < 0.001$
Incidental tumor tests	$p = 0.006$	$p = 0.080$	$p = 0.052$	$p = 0.022$
Female				
Hematopoietic hyperplasia (bone marrow)	3/49 (6%)	14/45 (31%)	8/50 (16%)	13/49 (26%)
Lymphoma, all malignant	15/49 (31%)	24/45 (53%)	24/50 (48%)	20/49 (41%)
Life table tests	$p = 0.031$	$p = 0.021$	$p = 0.025$	$p = 0.037$
Incidental tumor tests	$p = 0.446$	$p = 0.028$	$p = 0.109$	$p = 0.357$
Leukemia	0/49 (0%)	1/45 (2%)	2/50 (4%)	2/49 (4%)
Lymphoma or leukemia[b]	15/49 (31%)	25/45 (56%)	26/50 (52%)	22/49 (45%)
Life table tests	$p = 0.014$	$p = 0.014$	$p = 0.012$	$p = 0.017$
Incidental tumor tests	$p = 0.309$	$p = 0.014$	$p = 0.061$	$p = 0.272$

[a]Historical incidence at laboratory (mean ± SD): 13/100 (13%); historical incidence in NTP studies: 132/1090 (12% ± 6%).
[b]Historical incidence at laboratory (mean ± SD): 25/99 (25%); historical incidence in NTP studies: 258/1187 (22% ± 9%).

Table 19. Analysis of forestomach lesions in mice in the 2-year gavage studies of benzene.

Forestomach lesions	Vehicle control	25 mg/kg	50 mg/kg	100 mg/kg
Male				
Epithelial hyperplasia	1/45 (2%)	11/42 (26%)	2/44 (5%)	0/38 (0%)
Hyperkeratosis	1/45 (2%)	7/42 (17%)	4/44 (9%)	1/38 (3%)
Squamous cell papilloma	2/45 (4%)	1/42 (2%)	2/44 (5%)	5/38 (13%)
Life table tests	$p = 0.003$	$p = 0.567$	$p = 0.556$	$p = 0.014$
Incidental tumor tests	$p = 0.048$	$p = 0.546$	$p = 0.685$	$p = 0.161$
Squamous cell carcinoma	0/45 (0%)	1/42 (2%)	1/44 (2%)	1/38 (3%)
Squamous cell papilloma or carcinoma[a]	2/45 (4%)	2/42 (5%)	3/44 (7%)	5/38 (13%)
Life table tests	$p = 0.004$	$p = 0.623$	$p = 0.335$	$p = 0.014$
Incidental tumor tests	$p = 0.074$	$p = 0.640$	$p = 0.521$	$p = 0.161$
Female				
Epithelial hyperplasia	1/42 (2%)	3/40 (8%)	6/45 (13%)	6/42 (14%)
Hyperkeratosis	0/42 (0%)	1/40 (2%)	5/45 (11%)	9/42 (19%)
Squamous cell papilloma[b]	1/42 (2%)	3/40 (7%)	6/45 (13%)	5/42 (12%)
Life table tests	$p = 0.022$	$p = 0.288$	$p = 0.038$	$p = 0.040$
Incidental tumor tests	$p = 0.071$	$p = 0.134$	$p = 0.059$	$p = 0.079$

[a]Historical incidence at laboratory (mean): 0/100; historical incidence in NTP studies: 7/1055 (0.7%).
[b]Historical incidence at laboratory (mean): 0/99; historical incidence in NTP studies: 7/1077 (0.6%).

the number of neoplasms in vehicle controls remained the same (2/45) and the number in the dosed groups increased (from 8/124 to 10/124). The increased incidences of squamous cell papillomas and squamous cell papillomas or carcinomas (combined) in high-dose male mice were not statistically increased. The increased incidences of squamous cell papillomas in dosed females were also not statistically increased; no squamous cell carcinomas were observed. However, these neoplasms occur only rarely in control mice (< 1%).

Liver. In female mice, the incidences of hepatocellular adenomas in the low-dose group and hepatocellular adenomas or carcinomas (combined) in the low- and mid-dose groups were greater than those in the vehicle controls (Table 20).

Adrenal Gland. The incidences of hyperplasia in the adrenal capsule in dosed mice of each sex (except high-dose males) were greater than those in the vehicle controls (Table 21). The incidence of pheochromocytomas in mid-dose male mice was greater than that in the vehicle controls. In female mice, pheochromocytomas or malignant pheochromocytomas (combined) occurred with a negative trend, and the incidences in the dosed groups were lower than that in the vehicle controls.

Hematologic Analyses. Hematologic effects were limited to lymphocytopenia (data not shown). The analyses of variance indicate strong dose effects that varied across time for both male and female rats. For males, the data suggest a compound-related reduction in lymphocyte count in months 3 through 21. The significance of the interaction term in the analysis of variance for months 12 through 21 is reflected in temporal changes in the magnitude of differences between vehicle control and dosed groups. Overall, the pattern of response, without regard for magnitude of differences, is consistent across time, suggesting a compound-related depression of lympho-

Table 20. Analysis of liver tumors in mice in the 2-year gavage studies of benzene.

Liver neoplasms	Vehicle control	25 mg/kg	50 mg/kg	100 mg/kg
Male				
Hepatocellular adenoma	7/49 (14%)	11/48 (23%)	6/50 (12%)	3/47 (6%)
Life table tests	$p = 0.519$	$p = 0.108$	$p = 0.438$	$p = 0.518$
Incidental tumor tests	$p = 0.221$N[a]	$p = 0.131$	$p = 0.583$	$p = 0.519$N
Hepatocellular carcinoma	9/49 (18%)	8/48 (17%)	17/50 (34%)	8/47 (17%)
Life table tests	$p = 0.072$	$p = 0.589$	$p = 0.028$	$p = 0.293$
Incidental tumor tests	$p = 0.234$N	$p = 0.392$N	$p = 0.231$	$p = 0.215$N
Hepatocellular adenoma or carcinoma[b]	15/49 (31%)	17/48 (35%)	22/50 (44%)	11/47 (23%)
Life table tests	$p = 0.076$	$p = 0.256$	$p = 0.029$	$p = 0.225$
Incidental tumor tests	$p = 0.136$N	$p = 0.423$	$p = 0.238$	$p = 0.207$N
Female				
Hepatocellular adenoma	1/49 (2%)	8/44 (18%)	5/50 (10%)	4/49 (8%)
Life table tests	$p = 0.156$	$p = 0.008$	$p = 0.079$	$p = 0.077$
Incidental tumor tests	$p = 0.289$	$p = 0.008$	$p = 0.168$	$p = 0.124$
Hepatocellular carcinoma	3/49 (6%)	4/44 (9%)	8/50 (16%)	4/49 (8%)
Life table tests	$p = 0.169$	$p = 0.440$	$p = 0.058$	$p = 0.278$
Incidental tumor tests	$p = 0.401$	$p = 0.498$	$p = 0.101$	$p = 0.471$
Hepatocellular adenoma or carcinoma[c]	4/49 (8%)	12/44 (27%)	13/50 (26%)	7/49 (14%)
Life table tests	$p = 0.103$	$p = 0.014$	$p = 0.008$	$p = 0.086$
Incidental tumor test	$p = 0.339$	$p = 0.017$	$p = 0.026$	$p = 0.209$

[a]N = negative trend or decrease compared to controls.
[b]Historical incidence at laboratory (mean ± SD): 35/100 (35%); historical incidence in NTP studies: 340/1084 (31% ± 10%).
[c]Historical incidence at laboratory (mean ± SD): 6/98 (6%); historical incidence in NTP studies: 80/1176 (7% ± 3%).

Table 21. Analysis of adrenal gland lesions in mice in the 2-year gavage studies of benzene.

Adrenal gland lesions	Vehicle control	25 mg/kg	50 mg/kg	100 mg/kg
Male				
Hyperplasia (adrenal capsule)	2/47 (4%)	32/48 (67%)	14/49 (29%)	4/46 (9%)
Pheochromocytoma[a]	1/47 (2%)	1/48 (2%)	7/49 (14%)	1/46 (2%)
Life table tests	$p = 0.096$	$p = 0.725$	$p = 0.010$	$p = 0.632$
Incidental tumor tests	$p = 0.540$	$p = 0.757$N[b]	$p = 0.045$	$p = 0.529$N
Female				
Hyperplasia (adrenal capsule)	5/49 (10%)	19/44 (43%)	34/50 (68%)	30/48 (63%)
Pheochromocytoma	6/49 (12%)	1/44 (2%)	1/50 (2%)	1/48 (2%)
Life table tests	$p = 0.097$N	$p = 0.080$N	$p = 0.097$N	$p = 0.192$N
Incidental tumor tests	$p = 0.097$N	$p = 0.080$N	$p = 0.097$N	$p = 0.192$N
Pheochromocytoma, malignant	2/49 (4%)	0/44 (0%)	0/50 (0%)	0/48 (0%)
Pheochromocytoma or pheochromocytoma, malignant[c]	8/49 (16%)	1/44 (2%)	1/50 (2%)	1/48 (2%)
Life table tests	$p = 0.030$N	$p = 0.030$N	$p = 0.035$N	$p = 0.085$N
Incidental tumor tests	$p = 0.018$N	$p = 0.033$N	$p = 0.025$N	$p = 0.046$N

[a]Historical incidence at laboratory (mean ± SD): 6/100 (60%); historical incidence in NTP studies: 24/1037 (2.3% ± 2.6%)
[b]N = negative trend or decrease compared to controls.
[c]Historical incidence at laboratory (mean ± SD): 1/97 (1.0%); historical incidence in NTP studies: 11/1056 (1.0% ± 1.79%).

cytes in males. There is also evidence of a similar dose-related effect on lymphocytes in females. As in males, differences between dosed and vehicle control means occurred in months 3 through 21. However, that pattern of response is not as consistent or dramatic as that seen in the data for males.

Hematologic effects in mice were limited to lymphocytopenia as well. For both males and females, the analyses of variance suggest strong, temporally variable dose effects in the second year of the studies. Significant dose effects are also evident from males in the first year. The temporal variation in dose response in males appears to be mainly the result of time-oriented changes in the magnitude of difference between vehicle control and dosed groups. However, with the exception of month 12, there is consistent evidence of compound-related depression in lymphocytes in months 3 through 21, suggesting a compound effect in males. A higher degree of temporal variability in pattern of response makes a similar effect in females questionable.

The NTP found that benzene administered by gavage induced micronuclei in male and female B6C3F$_1$ mice; males were more sensitive than females (*97*).

A complete statistical analysis of all hematology data is available from the National Toxicology Program. However, the technical quality of certain of these data was considered questionable; thus, more detailed analyses (e.g., investigation of the association between hematologic and pathologic changes) are deemed inappropriate for these data.

Discussion

Design

Two-year toxicology and carcinogenicity studies of benzene (99.7% pure) were conducted on groups of 50 F344/N rats and 50 B6C3F$_1$ mice of each sex. Doses of 0, 50, 100, or 200 mg/kg benzene were administered to male rats by gavage in corn oil, 5 days per week for 103 weeks. Doses of 0, 25, 50, or 100 mg/kg were administered to female rats and mice of each sex on the same schedule. Doses for the 2-year studies were selected after evaluation of 17-week studies in which groups of 10 or 15 rats and 10 or 15 mice of each sex were administered 0, 25, 50, 100, 200, 400, or 600 mg/kg. The only clinical signs recorded for these 4-month studies were lowered body weights and ocular discharge in rats and tremors in mice in the higher dosed groups; some lymphoid depletion was observed in rats and leukopenia in mice. An additional 10 animals per group were started along with the 2-year animals; these were killed at 1 year. No biologically important toxic or carcinogenic lesions were observed, except possibly for the Harderian gland.

Body Weights and Survival

Mean body weights of some groups of dosed rats and mice were lower than those of the vehicle controls, and survival of some dosed groups was significantly lower than that of the corresponding vehicle controls. For male rats, the mean body weights after week 22 decreased with dose. The mean body weights of the high-dose male rats were notably lower than those of the vehicle controls, and mean body weights of mid- and high-dose male rats were slightly lower than those of the vehicle controls (Fig. 1). Mean body weights of high-dose female rats were slightly lower than those of the vehicle controls after week 62 (Fig. 2).

Survival decreased with increasing dose for both male and female rats (Tables 4 and 22, Figs. 5,6). Survival of the high-dose male rats and of the mid- and high-dose female rats was lower than that of the vehicle controls; survival in all groups of rats, except high-dose male rats, was 50% or more at 104 weeks. The reduced survival observed for the high-dose group of male rats (16/50, 32%) reflects to some degree a likely toxic response from the 200 mg/kg dose regimen, double the high dose used for the female rats and male and female mice (100 mg/kg). A partial reason for the apparent decrease in survival in dosed female rats is the greater-than-average survival seen for the vehicle controls (46/50, 92%). Although the survival of high-dose females was comparatively low (25/50, 50%), the numbers of animals alive at the end of the study for the low-dose (38/50, 76%) and the mid-dose (34/50, 68%) groups are considered to be good (*184,185*). In any event, survival at week 92 of all groups was 60% or more. Most rats that died early had neoplasia.

Mean body weights of mid- and high-dose male mice and high-dose female mice were lower than those of the vehicle controls (Figs. 3,4). Survival of high-dose male and female mice was lower than that of the vehicle controls (Tables 5 and 22, Figs. 7,8). The lower survival of the dosed groups of mice may have been a consequence of the increased incidences of life-threatening lymphomas and alveolar/bronchiolar carcinomas in the dosed groups rather than a singular toxic effect of benzene. At week 92, for instance, survival of all groups of mice was above 60%.

Hematology

Epidemiologic studies have shown that exposure of workers to benzene is associated with increased risk of aplastic anemia and of leukemia (*11,23,25,151-153, 161-169*). Benzene-associated leukopenia has been reported in mice, rats, and humans.

Benzene affected the hematopoietic system of F344/N rats and B6C3F$_1$ mice in the current 17-week studies; dose-related leukopenia, predominantly a lymphocytopenia, was observed for rats and mice of each sex, and compound-related lymphoid depletion and increased extramedullary hematopoiesis in the spleen were observed in rats of each sex.

For the 2-year studies, leukopenia was observed in dosed male rats during the early part of the study, and values returned to near control levels at the 21-month collection. In dosed female rats, leukopenia was evident between months 3 and 12. In male vehicle control mice, the white blood cell counts were greater than expected at the 6- and 9-month collections and could give an inaccurate

Table 22. Survival of F344/N rats and B6C3F$_1$ mice in the 2-year gavage studies of benzene.

Species/gender	Week	Vehicle control	Low dose	Mid dose	High dose
Rats					
Male	92	35/50 (70%)	38/50 (76%)	31/50 (62%)	30/50 (60%)
	104	32/50 (64%)	29/50 (58%)	25/50 (50%)	16/50 (32%)[a]
Female	92	49/50 (98%)	42/50 (84%)	40/50 (80%)	38/50 (76%)
	104	46/50 (92%)	38/50 (76%)	34/50 (68%)[a]	25/50 (50%)[a]
Mice					
Male	91	38/50 (76%)	34/50 (68%)	35/50 (70%)	32/50 (64%)
	104	28/50 (56%)	23/50 (46%)	18/50 (36%)	7/50 (14%)[a]
Female	91	39/50 (78%)	35/50 (70%)	40/50 (80%)	31/50 (62%)
	104	30/50 (60%)	26/50 (52%)	24/50 (48%)	18/50 (36%)[a]

[a]Decreased ($p < 0.05$) survival compared with vehicle controls.

impression of the compound-related leukopenia; similarly, high vehicle control group values for males were noted at 18 and 21 months. In dosed female mice, benzene-related leukopenia was observed at 12 and 18 months and lends support to the possibility that the decreases seen in the male mice might have been associated with benzene administration as well.

A dose-related lymphocytopenia was evident in male rats from 3 to 21 months. A similar, yet lesser effect was seen in female rats. For male mice, a decrease in lymphocytes was seen at the 3- through 9-month interval and during the 12- through 21-month period. The lymphocytopenia was evident for female mice between 12 and 18 months. Thus, exposure of rats and mice to benzene in these studies produced a mild to moderate leukocytopenia/lymphocytopenia. How this effect influences the other observed toxic and pathologic responses remains unknown.

Pathology

Increased incidences of neoplasms were observed at multiple sites for male rats, female rats, male mice, and female mice (Tables 23–26). Compound-related effects on the hematopoietic system, Zymbal gland, forestomach, and adrenal glands were found for both rats and mice.

Table 23. Incidences of selected nonneoplastic or neoplastic lesions in the 2-year gavage studies of benzene.

	Rats								Mice							
	Male				Female				Male				Female			
Organ/tissue lesion	Vehicle control	Low dose	Mid dose	High dose	Vehicle control	Low dose	Mid dose	High dose	Vehicle control	Low dose	Mid dose	High dose	Vehicle control	Low dose	Mid dose	High dose
Zymbal gland																
Hyperplasia	0/32	6/46	2/42	3/42	0/45	0/40	6/44	1/46	0/43	3/34	12/40	10/39	1/43	1/32	2/37	6/31
Carcinoma	2/32	6/46	10/42	17/42	0/45	5/40	5/44	14/46	0/43	1/34	4/40	21/39	0/43	0/32	1/37	3/31
Hematopoietic system																
Spleen, lymphoid depletion	0/49	19/48	8/47	23/47	0/50	11/50	8/49	10/49								
Bone marrow, hyperplasia									0/49	11/48	10/50	25/49	3/49	14/45	8/50	13/49
Malignant lymphoma									4/49	9/48	9/50	15/49	15/49	24/45	24/50	20/49
Forestomach																
Hyperkeratosis	2/48	4/44	3/48	9/47					1/45	7/42	4/44	0/38	0/42	1/40	5/45	7/42
Acanthosis	2/48	4/44	3/48	10/47												
Squamous cell papilloma									2/45	1/42	2/44	5/38	1/42	3/40	6/45	5/42
Squamous cell carcinoma									0/45	1/42	1/44	1/38				
Adrenal gland																
Zona fasciculata (focal hyperplasia)	0/50	13/49	0/48	2/49	0/50	15/50	0/47	0/49								
Hyperplasia of capsule									2/47	32/48	14/49	4/46	5/49	19/44	34/50	30/48
Pheochromocytoma									1/47	1/48	7/49	1/46	6/49	1/44	1/50	1/48
Oral cavity																
Squamous cell papilloma or carcinoma	1/50	9/50	16/50	19/50	1/50	5/50	12/50	9/50								
Lung																
Alveolar hyperplasia									2/49	3/48	7/50	10/49	1/49	1/42	9/50	6/49
A/B adenoma									6/49	6/48	8/50	12/49	4/49	2/42	5/50	9/49
A/B carcinoma									5/49	11/48	12/50	14/49	0/49	3/42	6/50	6/49
A/B adenoma or carcinoma									10/49	16/48	19/50	21/49	4/49	5/42	10/50	13/49
Skin																
Squamous cell papilloma or carcinoma	0/50	7/50	4/50	11/50												
Liver																
Adenoma	2/50	2/48	4/49	1/49	0/50	3/49	1/50	0/50	7/49	11/48	6/50	3/47	1/49	8/44	5/50	4/49
Adenoma or carcinoma	2/50	2/48	5/49	1/49					15/49	17/48	22/50	11/47	4/49	12/44	13/50	7/49
Harderian gland																
Focal hyperplasia									0/49	5/46	11/49	7/48	5/48	10/44	11/50	9/47
Adenoma									0/49	9/46	13/49	11/48	5/48	6/44	10/50	6/47
Adenoma or carcinoma									1/49	10/46	13/49	14/48	5/48	6/44	10/50	10/47
Preputial gland																
Hyperplasia									1/21	18/28	9/29	1/35				
Carcinomas									0/21	5/28	19/29	31/35				
Ovary																
Granulosa cell tumor													1/47	1/44	6/49	7/48
Benign mixed tumor													0/47	1/44	12/49	7/48
Tubular adenoma													0/47	1/44	3/49	3/48
Mammary gland																
Carcinoma													0/49	2/45	5/50	10/49
Carcinosarcoma													0/49	0/45	1/50	4/49

Table 24. Summary of primary neoplasms in F344/N rats and B6C3F$_1$ mice in the 2-year gavage studies of benzene.[a]

Organ/tissue site	Rats		Organ/tissue site	Mice	
	Male	Female		Male	Female
Zymbal gland	+	+	*Zymbal gland*	+	+
Oral cavity	+	+	Lymphoma	+	+
Skin	+	-	Lung	+	+
			Harderian gland	+	±
			Mammary gland	-	+
			Preputial gland	+	NA
			Forestomach	±	±
			Ovary	NA	+
			Liver	-	±

[a](+) increase relative to vehicle controls; (±) marginal increase relative to vehicle controls; (-) no difference from vehicle controls; NA, not applicable.

Table 25. Primary neoplasms in F344/N rats in the 2-year gavage studies of benzene.

Organ/tissue site	% of neoplasm-bearing animals			
	Vehicle control	Low dose	Mid dose	High dose
Male				
Oral cavity	2*	18+	32+	38+
Zymbal gland	6*	15	24+	43+
Skin	2*	14‡	10‡	24+
Female				
Oral cavity	2*	10‡	24+	18+
Zymbal gland	0*	13+	14+	33+
Uterus	14*	14	14	28+

*Dose-related trend ($p < 0.05$).
+Increased ($p < 0.05$) relative to vehicle controls.
‡Marginal increase ($p < 0.10$) relative to vehicle controls.

Table 26. Primary neoplasms in B6C3F$_1$ mice in the 2-year gavage studies of benzene.

Organ/tissue site	% of neoplasm-bearing animals			
	Vehicle control	Low dose	Mid dose	High dose
Male				
Zymbal gland	0*	3	10+	54+
Preputial gland	0*	18‡	66+	89+
Harderian gland	2*	22+	27+	29+
Lung	20*	33	38+	43+
Lymphoma/leukemia	8*	21+	20+	31+
Liver	31	35	44	23
Forestomach	4	5	7	13
Female				
Zymbal gland	0*	0	3	10+
Ovary	2*	8	47+	39+
Mammary gland	0*	4	12+	28+
Harderian gland	10*	14	20	21+
Lung	8*	12	20‡	27+
Lymphoma/leukemia	31*	56+	52+	45+
Liver	8	27+	26+	14
Forestomach	2	7	13‡	12‡

*Dose-related trend ($p < 0.05$).
+Increased ($p < 0.05$) relative to vehicle controls.
‡Marginal increase ($p < 0.10$) relative to vehicle controls.

The oral cavity was affected in rats only; and the lung, liver, Harderian gland, preputial gland, ovary, and mammary gland were affected in mice only.

Hematopoietic System. As was seen in the 17-week studies, lymphoid depletion of the spleen was observed at increased incidences in dosed male and female rats (see Table 23); lymphoid depletion of the thymus was seen in dosed male rats. Bone marrow hematopoietic hyperplasia was observed at increased incidences in dosed mice of each sex.

The incidences of malignant lymphomas in all dose groups of mice were greater than those of the vehicle controls. Benzene-associated neoplastic effects on the hematopoietic system were not observed in male or female rats. The incidences of mononuclear leukemia in dosed male rats were not different among dosed and vehicle control groups. In high-dose female rats, the incidence of mononuclear cell leukemia (9/50) was somewhat greater than that in the vehicle controls (6/50); this increase was significant by the life table test. These incidences are in the range observed in vehicle control female F344/N rats at the same laboratory (8/50–13/50) and therefore are not considered to be clearly related to administration of benzene. Further, the rates in concurrent vehicle controls are somewhat low compared with historical rates. Hydroquinone given by gavage in water at doses of 50 or 100 mg/kg body weight caused leukemia in female F344 rats (*142*). Phenol, the primary metabolite of benzene, increased the incidence of mononuclear cell leukemia in male F344/N rats that received 2500 ppm phenol in drinking water for 2 years, but this increase was not seen in male F344/N rats that received 5000 ppm (vehicle control, 18/50, 36%; low dose, 30/50, 60%; $p < 0.02$; high dose, 25/50, 50%) (*127,128*).

Zymbal Gland. Groups of modified sebaceous glands are located in the region of the external auditory canal in rats and mice and were originally described in 1933 by W. Zymbal and by C. Zawisch-Ossenitz apparently independently (*186*). Some see a correspondence between the sebaceous Zymbal gland and the modified sebaceous ceruminous gland in humans (glands in the skin of the external auditory canal that secrete the watery component of the cerumen); others consider this relation as tenuous. Yet Pliss (*186*) wrote that tumors of the auditory sebaceous glands in rats (and mice) are similar to tumors of the sebaceous glands in humans. Tumors of the auditory sebaceous (Zymbal) glands are uncommon (less than 1%) in F344/N rats and B6C3F$_1$ mice (*184*). These neoplasms usually arise at the base of the ear, often invading the ear canal, and have both sebaceous and squamous differentiation. Pohl and Fouts (*187*) found cytochrome P-450-dependent enzyme activity in homogenates of Zymbal glands from β-naphthoflavone-treated rats and mice. These authors suggested that chemical carcinogens can be metabolized to their initiating products in this gland.

The incidences of Zymbal gland carcinomas in the two upper dose groups of male rats and in all dose groups of female rats were greater than those in the vehicle controls (Tables 23–25) and exceeded the greatest incidences observed in corn oil vehicle historical controls. Epithelial

hyperplasia and carcinomas of the Zymbal gland were increased in mid- and high-dose male mice and in high-dose female mice. Because the Zymbal gland had been reported as a possible target organ (*188*), Zymbal glands from 10 animals per group were examined after 12 months of exposure; no nonneoplastic or neoplastic effects were observed.

Maltoni and Scarnato (*188*) first reported Zymbal gland tumors in female (but not male) Sprague-Dawley rats exposed to benzene at 50 mg/kg (two tumors in 30 rats) and 250 mg/kg (5/32); control rats in this colony reportedly had a background incidence of 0.9% (*189*). In later inhalation experiments, Maltoni et al. (*189*) reported increases in Zymbal gland carcinomas; incidences in Sprague-Dawley rats appear to be: male—control, 0.6%; 200 ppm, 1.4%; 300 ppm, 5.3%; female—control, 1.7%; 200 ppm, 1.7%; 300 ppm, 5.6%. Results from both inhalation and oral studies of Maltoni confirm the carcinogenic response of the Zymbal gland to benzene. Composite data from these studies are given in these proceedings (*126*).

Skin. Benzene was associated with neoplasms of the oral cavity and skin of rats but not of mice (Tables 23–25). The incidences of squamous cell papillomas and squamous cell carcinomas of the skin in high-dose male rats were greater than those observed in the concurrent or historical vehicle controls.

These neoplasms of the skin and oral cavity in rats exposed to benzene are unusual in that both adnexal and squamous elements are present. Even though these adenosquamous lesions contain sebaceous elements, they are distinct from the background sebaceous adenomas sometimes observed and appear to represent a benign form of an unusual benzene-related skin neoplasm. The skin neoplasms were considered to be a systemic effect of benzene metabolites, although the skin could have been topically exposed by the rats' grooming or by their exhaling unchanged benzene or its metabolites. Because benzene has been shown conclusively in numerous experiments not to induce skin tumors when applied topically, these skin lesions probably resulted from systemic exposure from reactive intermediates or from excretion of metabolites in saliva and then grooming. A less likely, yet possible, explanation for the oral lesions would be that intermediates are excreted by skin and thus the animals are exposed orally when they lick the skin. The uncommon squamous cell papillomas and squamous cell carcinomas of the oral cavity (including the palate, lip, and tongue) of dosed rats of each sex were probably due to a systemic effect of benzene-reactive intermediates. Squamous cell carcinomas of the oral cavity were also reported for Sprague-Dawley rats administered 500 mg/kg benzene by gavage for 104 weeks (*189*).

Lung. Administration of benzene was associated with alveolar epithelial hyperplasia in male and female mice, alveolar/bronchiolar carcinomas in male mice, alveolar/bronchiolar adenomas and alveolar/bronchiolar carcinomas in female mice (Tables 23, 24, and 26). The increased incidences of alveolar/bronchiolar neoplasms most likely result from a systemic effect of benzene rather than from a topical effect as a result of exhalation of unchanged benzene. Regardless of the route of exposure, benzene is apparently eliminated both in the expired air and in the urine (*28–31*). For instance, 40% of a single oral dose of benzene was reported to be exhaled unchanged from the lungs of rabbits (*190*); 70% of the benzene given SC to mice was found in expired air (*191*).

Harderian Gland. First described by J. Harder in the 1600s, Harderian glands are self-secreting accessory lacrimal glands at the inner corner of the eye in vertebrates that have well-developed nictitating membranes; these excrete an unctuous fluid that facilitates the movement of the third eyelid. These glands are rudimentary in humans (*192*). The Harderian gland is racemose and horseshoe shaped and lies deep within the orbit. The smaller arm lies superior to the larger arm, and these arms are connected by a narrow band. A single excretory duct opens at the base of the nictitating membrane (*193*). The color of the gland varies from pink to dark grey. The gland is thought to produce and excrete porphyrin.

Focal hyperplasia of the Harderian gland was observed at increased incidences in dosed mice of each sex (Table 23). The incidences of Harderian gland adenomas and adenomas or carcinomas (combined) in dosed male and in high-dose female mice were greater than those in the vehicle controls (Table 26). The incidences in the mid- and high-dose female mice were greater than those previously observed in corn oil vehicle controls (10/50 and 10/47 vs. 2/50); the incidence in the concurrent controls (5/48) is also greater than that previously observed.

Harderian glands were examined from the 10 animals per group killed at 12 months. Mild localized epithelial hyperplasia was observed in one female vehicle control, one low-dose male, and one mid-dose female; the mid-dose female had a small adenoma near the hyperplastic focus. One low-dose female had a small focus of necrosis and acute inflammation. One mid-dose male had a small adenoma.

Preputial Gland. Administration of benzene to male mice was associated with hyperplasia and squamous cell carcinomas of the preputial gland (Tables 23 and 26). The incidences of squamous cell carcinomas in the mid-dose (19/50) and high-dose (31/49) groups greatly exceed the overall historical incidence (1/1090). A possible explanation for this effect could be similar to that for the systemic-mediated skin and oral cavity lesions seen in rats. However, the potential effects of benzene exposure by grooming cannot be fully discounted. No increases were observed for this lesion in male rats. Likewise, no increase in neoplasia was observed in the clitoral gland of female mice or rats (histogenetically related to the preputial gland).

Ovary. Various uncommon nonneoplastic lesions and neoplastic lesions of the ovary (papillary cystadenoma, luteoma, granulosa cell tumors, tubular adenomas, benign mixed tumors, epithelial hyperplasia, and senile atrophy) were observed in benzene-exposed female mice (Tables 13, 14, 23, and 26). The incidences of granulosa cell tumors in the high-dose group and benign mixed tumors in mid- and high-dose groups of female mice were increased compared with those in the vehicle controls. Benign mixed

tumors and tubular adenomas of the ovary have not been reported previously in 1028 corn oil vehicle female control mice, whereas the historical incidence of granulosa cell tumors or carcinomas is 3/1028 as compared with 8/48 in the present study.

Mammary Gland. Incidences of carcinomas of the mammary gland in mid-dose and high-dose female mice and carcinosarcomas in high-dose female mice were increased (Tables 23 and 26). No comparable effects were seen in male mice or in male and female rats. Similarly, no increases in any nonneoplastic lesion of the mammary gland were seen in any group.

Forestomach. Hyperkeratosis and acanthosis in the nonglandular stomach were increased in high-dose male rats (Table 23). The incidences of hyperkeratosis in low-dose male mice and mid- and high-dose female mice were greater than those in the vehicle controls. Although the incidences of squamous cell papillomas in high-dose mice of each sex were not statistically greater than those in the vehicle controls, the incidences of these uncommon neoplasms in mice are noticeably greater than those observed in corn oil vehicle historical controls. No irritation response and no neoplastic effects were observed in rats. Thus, the increased incidences of squamous cell papillomas of the forestomach in mice were likely related to the administration of benzene (Table 26).

In a 1-year study, catechol given in the diet to male F344 rats at a concentration of 0.8% caused hyperplasias, adenomas, and adenocarcinomas of the glandular stomach and hyperplasia of the forestomach. After MNNG, catechol greatly enhanced both the forestomach and glandular stomach carcinogenesis. Mice were not studied (*139*).

Adrenal Gland. Focal hyperplasia of the zona fasciculata of the adrenal gland was observed at increased incidences in low-dose rats of each sex (Table 23). Hyperplasia of the adrenal capsule occurred at increased incidences in dosed mice of each sex. The incidence of pheochromocytomas in mid-dose male mice was greater than that in the vehicle concurrent and historical controls. In contrast, in dosed female mice, the incidence of pheochromocytomas was lower than that in the vehicle controls.

Liver. Heptocellular adenomas in low-dose female mice and hepatocellular adenomas or carcinomas (combined) in low- and mid-dose female mice were increased in comparison to those in the vehicle controls (Tables 23 and 26). These incidences were greater than those previously observed in corn oil vehicle controls. In male mice, the number of hepatocellular carcinomas in the mid-dose group was marginally higher than that in the vehicle controls by the life table test (9/49 versus 17/50) but was not statistically increased by the appropriate adjusted analysis (incidental tumor test). Kari (*142*) found similar gender differences in that hydroquinone caused liver neoplasms in female mice but no increases were observed for male mice. Maltoni et al. (*126,189*) reported that hepatocellular carcinomas were associated with inhalation exposure of Sprague-Dawley rats to benzene at 200 to 300 ppm for 4 to 7 hr/day for up to 104 weeks. In the current studies, the incidences of liver cell proliferative lesions were similar among dosed and vehicle control groups of F344/N rats; a slight increase was observed for clear cell changes in mid-dose male rats.

Summary

Carcinogenicity. Most of the increased incidences of neoplasia were quite evident, and the statistical significance did not depend on which test procedure was used. However, for other lesions, the findings were considered marginal. For example, certain neoplasms generally regarded as nonlethal showed significant increases by life table analysis but not by the more appropriate incidental tumor test; these neoplasms included squamous cell papillomas of the forestomach in male and female mice. Nonetheless, these neoplasms are relatively uncommon in B6C3F$_1$ mice (< 1%) and are probably associated with benzene administration; further, in both concurrent vehicle control groups, the incidences were male, 2/45; female, 1/42. In addition, some tumors showed apparent increases at lower doses which were not supported by similar high dose effects: hepatocellular adenomas or carcinomas in female mice and pheochromocytomas of the adrenal gland in male mice. Biologically, these might be considered as being possibly related to benzene exposure, given that the survival in the high-dose groups was uniformly lower than that in vehicle controls, and hence perhaps these groups could have had reduced sensitivity for exhibiting a carcinogenic response. Most of these animals died with either lung tumors or malignant lymphoma or both, and these competing risks could have reduced the likelihood of observing these late-appearing liver tumors. Also, pharmacokinetically, saturation of activation/deactivation systems do not allow consistent prediction as to which pathway will prevail. In any event, these tumors were considered not to be clearly due to benzene exposure.

The variety and multiplicity of toxic/carcinogenic responses to benzene exposure observed in these rodent studies raise the question of why the earlier 10 to 15 experiments [6 dermal, 4 inhalation, 4 injection (*11*)] failed to detect benzene-induced carcinogenicity. At least two possible explanations exit. Most of the earlier reported studies were less than adequate in comparison to current protocols and designs; deficiencies included, for example, too few animals, no control animals, short-duration, low-level exposures, and so on. In addition, because it was known that benzene caused hematotoxicity in rodents and humans (including leukemia), a number of long-term studies by design did not include or report complete pathologic results; the possible implication is that major or singular emphasis was placed on the hematopoietic system and lesser consideration was given to other organs or systems, thus accounting for the apparent lack of carcinogenic responses (or sensitivity) from rodents exposed to benzene.

More recent studies have been reported that collectively accumulate evidence that benzene is indeed carcinogenic to laboratory animals and, in particular, to rats

and mice. Individually, most of these studies tend to provide marginal or speculative evidence. Together with our data and the other relatively recent studies (*125,126*), the evidence for benzene carcinogenicity in animals is now unequivocal, convincing, and overwhelming: multiple site, multiple strain, and multiple species carcinogen.

Metabolism or Metabolites

Metabolism probably occurs most rapidly in the liver, where benzene is converted to benzene oxide, rearranges to form phenol, reacts with glutathione to form a premercapturic acid, or is hydrated to the dihydrodiol and then oxidized to catechol (Fig. 9). Hydroquinone is also formed from phenol. Various benzoquinones are formed from the two dihydroxy metabolites. A lesser pathway proposed by Parke and Williams (*190*) includes ring opening to muconaldehyde and muconic acid (*28,30,194*). Goldstein et al. offer the possibility of "ring expansion" to "benzene oxide-oxepin" (*195*). Considering that benzene apparently lacks direct-acting clastogenicity or carcinogenicity, together with the presence of carcinogenic responses of the metabolites studied so far (see "Introduction"), one could speculate with confidence that benzene metabolic derivatives possess or contribute to all the observed toxic/carcinogenic properties.

As early as 1949, Porteous and Williams (*196*) argued that the toxic effects of benzene are related to its metabolites, and the authors separated these into toxic (free phenols) and nontoxic (conjugates) phases. Since then, a number of studies have been published by Williams and co-workers on benzene and other chemicals (*196*). In addition, the bone marrow has been shown to convert benzene to its known metabolites (*197,198*). Irons et al. "clearly establish the capability of bone marrow to metabolize benzene independent of metabolism of the compound by the liver" (*198*). The authors stress, however, that recovered metabolites represented considerably less than 1% of the benzene administered to male F344/N rats. Certain benzene intermediates or combinations thereof surely represent the toxic moiety or (composite) of benzene.

Nonetheless, evidence exists that metabolic production of reactive intermediates is required for expressing benzene-induced toxicity and probably carcinogenicity as well. As yet, the "active" chemical or chemicals have not been identified conclusively. Two putative toxic metabolites (benzoquinone and muconaldehyde) have attracted the most attention. Further metabolism studies in which the doses administered are the same as those used in these current studies do permit better determination of altered metabolism—types and amounts of metabolites and pathways. The speculation that intermediates (or a cumulative metabolic effect) cause the observed benzene-associated toxicities remains to be further elucidated.

NIEHS has already made some interesting contributions to better understanding the multiplicative variables that influence the metabolism of benzene under various experimental conditions (routes of exposure and dosage regimens) using several species (*29–32*).

Genetic Toxicology

Rickert et al. showed that after rats were exposed to benzene, catechol, and hydroquinone were retained in the bone marrow at higher concentrations and for longer times than phenol (*199*). Catechol and hydroquinone (or their metabolites) can be concentrated in bone marrow and lymphoid organs (*200*), and reports suggest that the toxicity of benzene may be related to the concentration of catechol and hydroquinone in bone marrow (*26*).

The unique metabolism of benzene complicates studies performed *in vitro*. In addition, benzene is so volatile that one study showed that 90% of a 250-ppm solution was lost to the head space after 1 hr at 24°C; however, benzene did not appear to react with the culture medium with or without fetal calf serum (*201*).

Although catechol and hydroquinone can induce SCEs in human lymphocytes *in vitro*, benzene induced SCEs *in vitro* only after metabolic activation (*83,84*). In addition, Tice et al. (*102*) and Erexson et al. (*85*) have shown that benzene required metabolic transformation in order to induce SCEs. Erexson et al. (*85*) observed the following ranking of benzene and its metabolites for induction of SCEs in human lymphocytes *in vitro*: catechol > 1,4-benzoquinone > hydroquinone > 1,2,4-benzenetriol > benzene. Hydroquinone induced SCEs in CHO cells and in cultured human lymphocytes (*82,84*). In addition, hydroquinone also induced micronuclei in mice *in vivo* (*92,98,202,203*). As expected for a clastogen, hydroquinone was not mutagenic in Salmonella (*38,202,204*).

Catechol induced SCEs in cultured human lymphocytes (*82,84*), and chromosomal aberrations in CHO cells (*205*). Tunek et al. found that catechol failed to induce micronuclei in mice *in vivo* (*92*). Benzene and catechol induced DNA strand breaks in mouse lymphoma L5178Y cells (*206*). Consistent with the clastogenic activities of benzene and its metabolites in mammalian cells is the finding by Crebelli et al. that hydroquinone > catechol > phenol in producing chromosomal aberrations in the fungus *Aspergillus nidulans* (*207*), and free radicals appeared to be implicated in the clastogenic mechanisms of these metabolites.

Because human lymphocytes have mixed-function oxidases and can metabolize many phenolic compounds (*208,209*) and because benzene can be metabolized to these phenolic derivatives in bone marrow *in situ*, Tunek et al. (*210*) and Morimoto et al. (*84*) have suggested that compounds formed by further metabolism of catechol and hydroquinone might be responsible for the biological effects of benzene. These authors did not mention benzene oxide or other potential metabolic intermediates formed before the phenolic stage. Harper and Legator (*99*) have provided indirect evidence that benzene is activated in part by a cytochrome P-450 isozyme that is different from those that activate benzo[*a*]pyrene or cyclophosphamide.

Morimoto et al. (*84*) observed that rat liver S9 enhanced the ability of catechol and hydroquinone to induce SCEs in cultured human lymphocytes. Tunek et al. have shown that hydroquinone is further converted to *p*-benzosemiquinone and *p*-benzoquinone (*210,211*). These metabolites

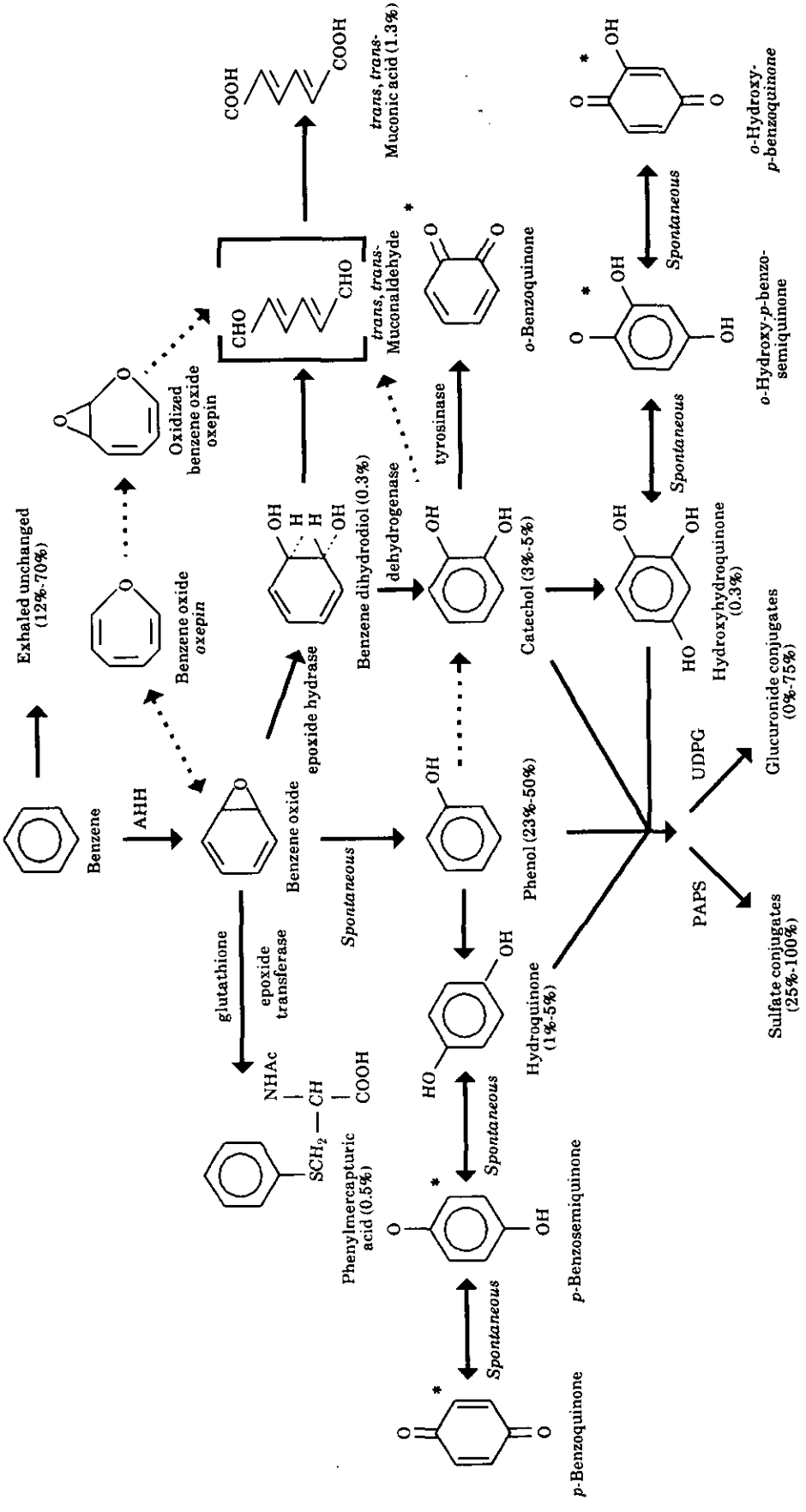

FIGURE 9. Pathways of benzene metabolism and excretion. Values in parentheses are percentages of metabolic products detected in urine of animals (rabbits, rats, mice, dogs) or humans. Asterisks denote putative or demonstrated alkylating activity toward intracellular nucleophiles, AHH, aryl hydrocarbon hydroxylase; UDPG, uridine diphosphate glucuronyltransferase; PAPS, 3′-phosphoadenosine-5′phosphosulfate. Dashed lines indicate putative pathways.

of hydroquinone, in addition to the metabolites of catechol (presumably *o*-benzoquinone and *o*-hydroxy-*p*-benzosemiquinone), may contribute to the genetic toxicity induced by benzene.

Numerous studies have documented a variety of chromosomal aberrations in humans who have benzene-associated myelotoxicity. Almost all humans with benzene-associated leukemia were exposed to other chemicals, although exposure to benzene was common to all. The possibility exists, albeit equivocally, that other chemicals in addition to benzene may have been involved in the development of the leukemia, and this possibility could help explain the failure to reproduce benzene-associated leukemia is the present NTP studies or in other such studies in experimental animals (*212*); nonetheless, benzene caused lymphomas in both sexes of mice in the NTP study. Cronkite et al. reported an increase in lymphomas in female C57BL/6 mice exposed at 300 ppm for 16 weeks and observed until death (*125,213,214*). These authors suggest that prolonged exposures may suppress the incidence of lymphomas or shorten the lifespan of mice, so lymphomas cannot be observed. Even so, perhaps rodents are not adequate models for human leukemogenesis.

In this regard, Post et al. found that *p*-benzoquinone inhibited T-cell growth factor interleukin-2 (IL-2), and benzene and *p*-benzoquinone inhibited RNA synthesis in mouse spleen lymphocytes at concentrations that had no significant effect on lymphocyte viability (*215*). Thus, the inhibition of RNA synthesis in lymphocytes by benzene may prevent the production of factors (IL-2) required for hematopoiesis and contribute to the aplastic anemia caused by benzene. Similarly, benzoquinone was the most potent inhibitor of DNA synthesis in mouse L5178Y cells followed by hydroquinone, benzenetriol, catechol, and phenol (*216*). In addition, DNA adducts were formed, isolated, and partially characterized from rat liver mitoplasts incubated with benzene (*217*).

The malignant cells of most neoplasia, including lymphomas and leukemias, have chromosomal abnormalities, and for most, specific chromosomal defects are associated with specific neoplasia (*218*). Thus, it is not surprising that benzene induced fragile sites in cultured human lymphocytes and that these sites correlated with chromosomal break points known to occur in cancer cells (*219*).

Interestingly, benzene (or metabolites) causes cytogenetic abnormalities, such as chromosomal aberrations, SCEs, and micronuclei, but does not cause gene mutations. Most of the positive results obtained with benzene have been obtained from *in vivo* systems in which cytogenetic end points have been measured. It will be interesting to learn if benzene is positive in a somatic-cell, gene-mutation assay *in vivo*. If it is not, then benzene will have the distinction of being one of the few chemicals known that is solely a clastogen (an agent that breaks chromosomes) but does not cause gene mutations. If benzene is capable of inducing gene mutations *in vivo*, then the disparity between the positive *in vivo* cytogenetic results and the negative *in vitro* gene mutation results would be explained by the fact that reactive metabolites of benzene are formed *in vivo*.

In summary, benzene has been long recognized as a hazardous chemical in the workplace and to a lesser extent in the general environment. Definitive evidence now exists that benzene induces multiple-site neoplasia in laboratory rodents. This logically led to the forecast that benzene would likely be associated with other tumor site increases in humans as well (*25*). Unfortunately, this has now been shown to be true (*168,169*).

A positive association has been established between occupational exposure to benzene and aplastic anemia and acute myelogenous leukemia in workers, and as recorded in these proceedings (*169*), benzene has now been associated with several other tumor sites in humans (*124*). Now that catechol has been shown to be a potent carcinogen for the forestomach of male rats (*139*) and because there appear to be increases in leukemia in female rats and in liver neoplasia in mice (primarily female) exposed to hydroquinone (*142*), one can begin to better understand the multiplicity of responses observed in the present studies (Tables 23-26). Benzene most likely undergoes metabolism to several active metabolites, each of which might be associated with one or more of the site-specific neoplasms induced. When the target site, for instance the hematopoietic system, for two metabolites is the same, then both of these (hydroquinone and phenol) could be considered to influence the response. Thus, if one hypothesizes that each of several metabolites may exert a carcinogenic effect in separate (or the same) target organs, this may explain the pancarcinogenesis of benzene. Given the number of potentially active metabolites (Fig. 5), and knowing that some have already been shown to cause neoplasia in independent studies, then the possibility gains strength that these metabolites influence different site specificities.

Further, benzene has induced a larger number of unique sites of neoplasia than any other of the nearly 375 chemicals studied under the aegis of the National Cancer Institute or the National Toxicology Program (*3-6*). Whether several or all metabolites (alone or in combination with benzene) induce the toxic, clastogenic, and carcinogenic effects awaits further definitive study. Meanwhile the OSHA has promulgated a 10-fold lower permissible occupational exposure level, from 10 ppm to 1 ppm (*25*) to better protect public health.

Conclusions

Under the conditions of these 2-year gavage studies, there was clear evidence of carcinogenicity of benzene for male F344/N rats, for female F344/N rats, for male $B6C3F_1$ mice, and for female $B6C3F_1$ mice. For male rats, benzene caused increased incidences of Zymbal gland carcinomas, squamous cell papillomas and squamous cell carcinomas of the oral cavity, and squamous cell papillomas and squamous cell carcinomas of the skin. For female rats, benzene caused increased incidences of Zymbal gland carcinomas and squamous cell papillomas and squamous cell carcinomas of the oral cavity. For male

mice, benzene caused increased incidences of Zymbal gland squamous cell carcinomas, malignant lymphomas, alveolar/bronchiolar carcinomas and alveolar/bronchiolar adenomas or carcinomas (combined), Harderian gland adenomas, and squamous cell carcinomas of the preputial gland. For female mice, benzene caused increased incidences of malignant lymphomas, ovarian granulosa cell tumors, ovarian benign mixed tumors, carcinomas and carcinosarcomas of the mammary gland, alveolar/bronchiolar adenomas, alveolar/bronchiolar carcinomas, and Zymbal gland squamous cell carcinomas.

We appreciate the contributions made to this paper by the members of three Pathology Working Groups (Chairpersons: G. Boorman, S. Eustis, R. Maronpot), by the NTP Board of Scientific Counselor's Peer Review Panel (Chairperson: J. Hook), and by C. Alden, J. Bucher, L. G. Hart, A. Noles, C. Robbins, and S. Soward.

REFERENCES

1. Huff, J. E. Toxicology and Carcinogenesis Studies of Benzene (CAS No. 71-43-2) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies). Technical Report No. 289. DHHS, National Toxicology Program/National Institute of Environmental Health Sciences, Research Triangle Park, NC (1986).
2. Huff, J. E., Eastin, W., Roycroft, J., Eustis, S. L., and Haseman, J. K. Carcinogenesis studies of benzene, methyl benzene, and dimethyl benzenes. Ann. N. Y. Acad. Sci. 534: 427–440 (1988).
3. Chu, K. C., Cueto, C., Jr., and Ward, J. M. Factors in the evaluation of 200 National Cancer Institute carcinogen bioassays. J. Toxicol. Environ. Health 8: 251–280 (1981).
4. Griesemer, R. A., and Cueto, C. Toward a classification scheme for degrees of experimental evidence for the carcinogenicity of chemicals for animals. In: Molecular and Cellular Aspects of Carcinogen Screening Tests, 27 (R. Montesano, H. Bartsch, and L. Tomatis, Eds.), IARC Scientific Publications, International Agency for Research on Cancer, Lyon, France, 1986, pp. 259–281.
5. Haseman, J. K., Huff, J. E., Zeiger, E., and McConnell, E. E. Comparative results of 327 chemical carcinogenicity studies. Environ. Health Perspect. 74: 229–235 (1987).
6. Huff, J. E., McConnell, E. E., Haseman, J. K., Boorman, G. A., Eustis, S. L., Schwetz, B. A., Rao, G. N., Jameson, C. W., Hart, L. G., and Rall, D. P. Carcinogenesis studies: results of 398 experiments on 104 chemicals from the U. S. National Toxicology Program. Ann. N. Y. Acad. Sci. 534: 1–30 (1988).
7. Chemical and Engineering News. Top 50 chemicals production reaches record high. April 10, 1989, pp. 11–15.
8. Purcell, W. Benzene. In: Kirk-Othmer Encyclopedia of Chemical Technology, Vol. 3, 3rd ed. John Wiley & Sons, New York, 1978, pp. 744–771.
9. Chemical and Engineering News. Key Chemicals. Benzene. November 17, 1980, p. 20.
10. Criteria Group for Occupational Standards (CGOS). Scientific basis for Swedish occupational standards. Arbete och Halsa 9: 24–37 (1982).
11. IARC. Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans: Some Industrial Chemicals and Dyestuffs, Vol. 29. International Agency for Research on Cancer, Lyon, France, 1982, pp. 95–148.
12. Brief, R. S., Lynch, J., Bernath, T., and Scala, R. A. Benzene in the workplace. Am. Ind. Hyg. Assoc. J. 41: 616–623 (1980).
13. Hunter, D. The Diseases of Occupations, 6th ed. Hodder and Stoughton, London, 1980, pp. 489–499.
14. Selling, L. Benzol as a leucotoxin. Studies on the degeneration and regeneration of the blood and hematopoietic organs. Johns Hopkins Hosp. Rep. 17: 83–142 (1916).
15. National Institute for Occupational Safety and Health (NIOSH). Occupational Diseases. A Guide to Their Recognition. U. S. Department of Health, Education, and Welfare, Washington, DC, 235–238 (1977).
16. National Research Council (NRC). Drinking Water and Health, Vol. 3. National Academy of Sciences, Washington, DC, pp. 80–86, 261–262 (1980).
17. Lauwerys, R. Industrial Health and Safety. Human Biological Monitoring of Industrial Chemicals. 1. Benzene. Commission of the European Communities, Luxembourg (1979).
18. Dowty, B., Carlisle, D., and Laseter, J. New Orleans drinking water sources tested by gas chromatography-mass spectrometry. Occurrence and origin of aromatics and halogenated aliphatic hydrocarbons. Environ. Sci. Technol. 9: 762–765 (1975).
19. Chang, S., and Peterson, R. Symposium: The basis of quality in muscle foods. Recent developments in the flavor of meat. J. Food Sci. 42: 298–305 (1977).
20. Environmental Protection Agency (EPA). National emission standards for hazardous air pollutants; benzene emissions from maleic anhydride plants, ethylbenzene/styrene plants, benzene storage vessels, benzene equipment leaks, and coke by-product recovery plants: proposed rule and notice of public hearing. Fed. Register 53 (145): 28496–28592 (28 July 1988).
21. Susten, A. S., Dames, B. L., Burg, J. R., Neimeier, R. W. Percutaneous penetration of benzene in hairless mice: an estimate of dermal absorption during tire-building operations. Am. J. Ind. Med. 7: 323–335 (1985).
22. Blank, I. H., and McAuliffe, D. J. Penetration of benzene through human skin. J. Invest. Derm. 85: 522–526 (1985).
23. Occupational Safety and Health Administration (OSHA). Occupation exposure to benzene: proposed rule and notice of public hearing. Fed. Register 50 (237): 50512–50586 (10 December 1985).
24. Holmberg, B., and Lundberg, P. Benzene: Standards, occurrence, and exposure. Am. J. Ind. Med. 7: 375–383 (1985).
25. Occupational Safety and Health Administration (OSHA). Occupational exposure to benzene; final rule. 52 (176): 34460–34578 (11 September 1987).
26. Irons, R., and Pfeifer, R. Benzene metabolites: evidence for an epigenetic mechanism of toxicity. Environ. Sci. Res. 25: 241–256 (1982).
27. Bolcsak, L. E., and Nerland, D. E. Inhibition of erythropoiesis by benzene and benzene metabolites. Toxicol. Appl. Pharmacol. 69: 363–368 (1983).
28. Rusch, G. M., Leong, B. K., and Laskin, S. Benzene metabolism. J. Toxicol. Environ. Health (Suppl.) 2: 23–36 (1977).
29. Sabourin, P. J., Chen, B. T., Lucier, G., Birnbaum, L. S., Fisher, E., and Henderson, R. F. Effect of dose on the absorption and excretion of [14C] benzene administered orally or by inhalation in rats and mice. Toxicol. Appl. Pharmacol. 87: 325–336 (1987).
30. Sabourin, P. J., Bechtold, W. E., Birnbaum, L. S., Lucier, G., and Henderson, R. F. Differences in the metabolism of inhaled [^{3}H] benzene by F344/N rats and B6C3F$_1$ mice. Toxicol. Appl. Pharmacol. 94: 128–140 (1988).
31. Henderson, R. F., Sabourin, P. J., Bechtold, W. E., Griffith, W. C., Medinsky, M. A., Birnbaum, L. S., and Lucier, G. W. The effect of dose, dose rate, route of administration and species on tissue and blood levels of benzene metabolism. Environ. Health Perspect. 82: 9–17 (1989).
32. Medinsky, M. A., Sabourin, P. J., Henderson, R. F., Lucier, G., and Birnbaum, L. S. Differences in the pathways for metabolism of benzene in rats and mice simulated by a physiological model. Environ. Health Perspect. 82: 43–49 (1989).
33. Dean, B. J. Recent findings on the genetic toxicology of benzene, toluene, xylenes and phenols. Mutat. Res. 154: 153–181 (1985).
34. Kalf, G. F. Recent advances in the metabolism and toxicity of benzene. CRC Crit. Rev. Toxicol. 18: 141–159 (1987).
35. Simmon, V., Kauhanen, K., and Tardiff, R. Mutagenic activity of chemicals identified in drinking water. Dev. Toxicol. Environ. Sci. 2: 249–258 (1977).
36. Cotruvo, J. A., Simmon, V. F., and Spanggord, R. J. Investigation of mutagenic effects of products of ozonation reactions in water. Ann. N. Y. Acad. Sci. 298: 124–140 (1977).
37. Shahin, M. M., and Fournier, F. Suppression of mutation induction and failure to detect mutagenic activity with Athabasca tar sand fractions. Mutat. Res. 58: 29–34 (1978).

38. Florin, I., Rutberg, L., Curvall, M., and Enzell, C. R. Screening of tobacco smoke constituents for mutagenicity using the Ames' test. Toxicology 15: 219-232 (1980).
39. Nestmann, E. R., Lee, E. G., Matula, T. I., Douglas, G. R., and Mueller, J. C. Mutagenicity of constituents identified in pulp and paper mill effluents using the Salmonella/mammalian-microsome assay. Mutat. Res. 79: 203-212 (1980).
40. Ho, C. H., Clark, B. R., Guerin, M. R., Barkenbus, B. D., Rao, T. K., and Epler, J. L. Analytical and biological analyses of test materials from the synthetic fuels technologies. IV. Studies of chemical structure mutagenic activity relationships of aromatic nitrogen compounds relevant to synfuels. Mutat. Res. 85: 335-345 (1981).
41. Hermann, M. Synergistic effects of individual polycyclic aromatic hydrocarbons on the mutagenicity of their mixture. Mutat. Res. 90: 399-409 (1981).
42. Shimizu, M., Yasui, Y., and Matsumoto, N. Structural specificity of aromatic compounds with special reference to mutagenic activity in *Salmonella typhimurium*: a series of chloro- or fluoronitrobenzene derivatives. Mutat. Res. 116: 217-238 (1983).
43. Brams, A., Buchet, J. P., Crutzen-Fayt, M. C., De Meester, C., Lauwerys, R., and Lëonard, A. A comparative study, with 40 chemicals, of the efficiency of the Salmonella assay and the SOS chromotest (kit procedure). Toxicol. Lett. 38: 123-133 (1987).
44. Ames, B. N., McCann, J., and Yamasaki, E. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutat. Res. 31: 347-364 (1975).
45. Lyon, J. Mutagenicity studies with benzene (abstract). Diss. Abstr. Int. B. 36: 5537 (1975).
46. Bartsch, H., Malaveille, C., Camus, A., Martel-Planche, G., Brun, G., Hautefeuelle, A., Sabadie, N., Barbin, A., Kuroki, T., Drevon, C., Piccoli, C., and Montesano, R. Validation and comparative studies of 180 chemicals with *S. typhimurium* strains and V79 Chinese hamster cells in the presence of various metabolizing systems. Mutat. Res. 76: 1-50 (1980).
47. Bos, R. P., Theuws, J. L. G., Jongeneelen, F. J., and Henderson, P. T. Mutagenicity of bi-, tri-, and tetracyclic aromatic hydrocarbons in the "taped-plate assay" and in the conventional Salmonella mutagenicity assay. Mutat. Res. 204: 203-206 (1988).
48. Kaden, D. A., Hites, R. A., and Thilly, W. G. Mutagenicity of soot and associated polycyclic aromatic hydrocarbons to *Salmonella typhimurium*. Cancer Res. 39: 4152-4159 (1979).
49. Venitt, S. Summary report of the performance of the bacterial mutation assays. In: Evaluation of Short-Term Tests for Carcinogens: Report of the International Program of Chemical Safety Collaborative Study on In Vitro Assays (J. Ashby, F. J. deSerres, M. Draper, M. Ishidate, Jr., B. H. Margolin, B. E. Matter, and M. D. Shelby, Eds.), Elsevier, Amsterdam, 1985, pp. 11-23.
50. Tanooka, H. Development and application of *Bacillus subtilis* test systems for mutagens, involving DNA-repair deficiency and suppressible auxotrophic mutations. Mutat. Res. 42: 19-32 (1977).
51. McCarroll, N., Keech, B., and Piper, C. A microsuspension adaptation of the Bacillus subtilis "rec" assay. Environ. Mutagen. 3: 607-616 (1981).
52. McCarroll, N. E., Piper, C. E., and Keech, B. H. An E. coli microsuspension assay for the detection of DNA damage induced by direct-acting agents and promutagens. Environ. Mutagen. 3: 429-444 (1981).
53. Rosenkranz, H., and Leifer, Z. Determining the DNA-modifying activity of chemicals using DNA-polymerase-deficient *Escherichia coli*. In: Chemical Mutagens: Principles and Methods for Their Detection, Vol. 6 (F. deSerres and A. Hollaender, Eds.), Plenum Publishers, New York, 1980, pp. 109-147.
54. Nakamura, S.-I., Oda, Y., Shimada, T., Oki, I., and Sugimoto, K. SOS-inducing activity of chemical carcinogens and mutagens in *Salmonella typhimurium* TA1535/pSK1002: examination with 151 chemicals. Mutat. Res. 192: 239-246 (1987).
55. Egilsson, V., Evans, I. H., and Wilkie, D. Toxic and mutagenic effects of carcinogens on the mitochondria of *Saccaromyces cerevisiae*. Mol. Gen. Genet. 174: 39-46 (1979).
56. Parry, J. M. Summary report on the performance of the yeast and Aspergillus assays. In: Evaluation of Short-Term Tests for Carcinogens: Report of the International Program on Chemical Safety Collaborative Study on In Vitro Assays (J. Ashby, F. J. deSerres, M. Draper, M. Ishidate, Jr., B. H. Margolin, B. E. Matter, and M. D. Shelby, Eds.), Elsevier, Amsterdam, 1985, pp. 25-46.
57. Parry, J. M., and Eckardt, F. The induction of mitotic aneuploidy, point mutation and mitotic crossing-over in the yeast. *Saccharomyces cerevisiae* strains D61-M and D6. In: Evaluation of Short-Term Tests for Carcinogens: Report of the International Program on Chemical Safety Collaborative Study on In Vitro Assays (J. Ashby, F. J. deSerres, M. Draper, M. Ishidate, Jr., B. H. Margolin, B. E. Matter, and M. D. Shelby, Eds.), Elsevier, Amsterdam, 1985, pp. 261-269.
58. Nylander, P.-O., Olofsson, H., Rasmuson, B., and Svahlin, H. Mutagenic effects of petrol in *Drosophila melanogaster* I. Effects of benzene and 1,2-dichloroethane. Mutat. Res. 57: 163-167 (1978).
59. Würgler, F. E., Graf, U., and Frei, H. Somatic mutation and recombination test in wings of *Drosophila melanogaster*. In: Evaluation of Short-Term Tests for Carcinogens: Report of the International Program on Chemical Safety Collaborative Study on In Vitro Assays (J. Ashby, F. J. deSerres, M. Draper, M. Ishidate, Jr., B. H. Margolin, B. E. Matter, and M. D. Shelby, Eds.), Elsevier, Amsterdam, 1985, pp. 325-340.
60. Kale, P. G., and Baum, J. W. Genetic effects of benzene in *Drosophila melanogaster* males. Environ. Mutagen. 5: 223-226 (1983).
61. Fujikwawa, K., Ryo, H., and Kondo, S. The Drosophila reversion assay using the *zeste-white* somatic eye colour system. In: Evaluation of Short-Term Tests for Carcinogens: Report of the International Program on Chemical Safety Collaborative Study on In Vitro Assays (J. Ashby, F. J. deSerres, M. Draper, M. Ishidate, Jr., B. H. Margolin, B. E. Matter, and M. D. Shelby, Eds.), Elsevier, Amsterdam, 1985, pp. 319-324.
62. Vogel, E. W. The Drosophila somatic recombination and mutation assay using the white-coral somatic eye colour system. In: Evaluation of Short-Term Tests for Carcinogens: Report of the International Program on Chemical Safety Collaborative Study on In Vitro Assays (J. Ashby, F. J. deSerres, M. Draper, M. Ishidate, Jr., B. H. Margolin, B. E. Matter, and M. D. Shelby, Eds.), Elsevier, Amsterdam, 1985, pp. 313-317.
63. Schairer, L., Van't Hof, J., Haynes, C., Burton, R., and deSerres, F. Measurement of biological activity of ambient air mixtures using a mobile laboratory for *in situ* exposures: preliminary results from the *Tradescantia* plant test system. In: Application of Short-Term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures (M. Waters, M. D. Walters, S. Nesnow, J. L. Huising, S. S. Sandhu, and L. Claxton, Eds.), Environmental Protection Agency/600/9-78-027, 1978.
64. Schairer, L., and Sautkulis, R. Detection of ambient levels of mutagenic atmospheric pollutants with the higher plant Tradescantia. In: Environment Mutagenesis, Carcinogenesis, and Plant Biology, Vol. II (E. Klekowski, Ed.), Praeger, 1982, pp. 154-194.
65. Lyang, J. C., Hsu, T. C., and Henry, J. E. Cytogenetic assays for mitotic poisons: the grasshopper embryo system for volatile liquids. Mutat. Res. 113: 467-479 (1983).
66. Lebowitz, H., Brusick, D., Matheson, D., Jagannath, D., Reed, M., Goode, S., and Roy, G. Commonly used fuels and solvents evaluated in a battery of short-term bioassays. Environ. Mutagen. 1: 172-173 (1979).
67. Garner, R. C. Summary report on the performance of gene mutation assays in mammalian cells in culture. In: Evaluation of Short-Term Tests for Carcinogens: Report of the International Program on Chemical Safety Collaborative Study on In Vitro Assays (J. Ashby, F. J. deSerres, M. Draper, M. Ishidate, Jr., B. H. Margolin, B. E. Matter, and M. D. Shelby, Eds.), Elsevier, Amsterdam, 1985, pp. 85-94.
68. Probst, G. S., McMahon, R. E., Hill, L. E., Thompson, C. Z., Epp, J. K., and Neal, S. B. Chemically-induced unscheduled DNA synthesis in primary rat hepatocyte cultures: A comparison with bacterial mutagenicity using 218 compounds. Environ. Mutagen. 3: 11-32 (1981).
69. Probst, G. S., and Hill, L. E. Tests for the induction of DNA repair synthesis in primary cultures of adult rat hepatocytes. In: Evaluation of Short-Term Tests for Carcinogens: Report of the International Program on Chemical Safety Collaborative Study on In Vitro Assays (J. Ashby, F. J. deSerres, M. Draper, M. Ishidate, Jr., B. H.

Margolin, B. E. Matter, and M. D. Shelby, Eds.), Elsevier, Amsterdam, 1985, pp. 381-386.
70. Williams, G. M., Tong, C., and Brat, S. V. Tests with the rat hepatocyte primary culture/DNA repair test. In: Evaluation of Short-Term Tests for Carcinogens: Report of the International Program on Chemical Safety Collaborative Study on In Vitro Assays (J. Ashby, F. J. deSerres, M. Draper, M. Ishidate, Jr., B. H. Margolin, B. E. Matter, and M. D. Shelby, Eds.), Elsevier, Amsterdam, 1985, pp. 341-345.
71. Barrett, R. H. Assays for unscheduled DNA synthesis in HeLa 53 cells. In: Evaluation of Short-Term Tests for Carcinogens: Report of the International Program on Chemical Safety Collaborative Study on In Vitro Assays (J. Ashby, F. J. deSerres, M. Draper, M. Ishidate, Jr., B. H. Margolin, B. E. Matter, and M. D. Shelby, Eds.), Elsevier, Amsterdam, 1985, pp. 347-352.
72. Martin, C. N., and Campbell, J. Tests for the induction of unscheduled DNA synthesis in HeLa cells. In: Evaluation of Short-Term Tests for Carcinogens: Report of the International Program on Chemical Safety Collaborative Study on In Vitro Assays (J. Ashby, F. J. deSerres, M. Draper, M. Ishidate, Jr., B. H. Margolin, B. E., Matter, and M. D. Shelby, Eds.), Elsevier, Amsterdam, 1985, pp. 375-379.
73. Glauert, H. P., Kennan, W. S., Sattler, G. S., and Pitot, H. C. Assays to measure the induction of unscheduled DNA synthesis in cultured hepatocytes. In: Evaluation of Short-Term Tests for Carcinogens: Report of the International Program on Chemical Safety Collaborative Study on In Vitro Assays (J. Ashby, F. J. deSerres, M. Draper, M. Ishidate, Jr., B. H. Margolin, B. E. Matter, and M. D. Shelby, Eds.), Elsevier, Amsterdam, 1985, pp. 371-373.
74. Pellack-Walker, P., and Blumer, J. L. DNA damage in L5178YS cells following exposure to benzene metabolites. Mol. Pharmacol. 30: 42-47 (1986).
75. Koizumi, A., Dobashi, Y., Tachibana, Y., Tsuda, K., and Katsunuma, H. Cytokinetic and cytogenetic changes in cultured human leukocytes and Hela cells induced by benzene. Ind. Health 12: 23-29 (1974).
76. Morimoto, K. Combined cytogenetic effects of benzene and radiation on cultured human lymphocytes. Jpn. J. Ind. Health 17: 106-107 (1974).
77. Howard, C. A., Sheldon, T., and Richardson, C. R. Chromosomal analysis of human lymphocytes exposed in vitro to five chemicals. In: Evaluation of Short-Term Tests for Carcinogens: Report of the International Program on Chemical Safety Collaborative Study on In Vitro Assays (J. Ashby, F. J. deSerres, M. Draper, M. Ishidate, Jr., B. H. Margolin, B. E. Matter, and M. D. Shelby, Eds.), Elsevier, Amsterdam, 1985, pp. 457-467.
78. Gerner-Smidt, P., and Friedrich, U. The mutagenic effect of benzene, toluene, and xylene studied by the SCE technique. Mutat. Res. 58: 313-316 (1978).
79. Ishidate, M., Jr., and Sofuni, T. The in vitro chromosome aberration test using Chinese hamster lung (CHL) fibroblast cells in culture. In: Evaluation of Short-Term Tests for Carcinogens: Report of the International Program on Chemical Safety Collaborative Study on In Vitro Assays (J. Ashby, F. J. deSerres, M. Draper, M. Ishidate, Jr., B. H. Margolin, B. E. Matter, and M. D. Shelby, Eds.), Elsevier, Amsterdam, 1985, pp. 427-432.
80. Palitti, F., Fiore, M., DeSalvia, R., Tanzarella, C., Ricordy, R., Forster, R., Mosesso, P., Astolfi, S., and Loprieno, N. Chromosome aberration assays of 5 chemicals in Chinese hamster cells In vitro. In: Evaluation of Short-Term Tests for Carcinogens: Report of the International Program on Chemical Safety Collaborative Study on in Vitro Assays (J. Ashby, F. J. deSerres, M. Draper, M. Ishidate, Jr., B. H. Margolin, B. E. Matter, and M. D. Shelby, Eds.), Elsevier, Amsterdam, 1985, pp. 443-450.
81. Dean, B. J. Summary report on the performance of cytogenetic assays in cultured mammalian cells. In: Evaluation of Short-Term Tests for Carcinogens: Report of the International Program on Chemical Safety Collaborative Study on In Vitro Assays (J. Ashby, F. J. deSerres, M. Draper, M. Ishidate, Jr., B. H. Margolin, B. E. Matter, and M. D. Shelby, Eds.), Elsevier, Amsterdam, 1985, pp. 69-83.
82. Morimoto, K., and Wolff, S. Increase of sister chromatid exchanges and perturbations of cell division kinetics in human lymphocytes by benzene metabolites. Cancer Res. 40: 1189-1193 (1980).
83. Morimoto, K. Induction of sister chromatid exchanges and cell division delays in human lymphocytes by microsomal activation of benzene. Cancer Res. 43: 1130-1334 (1983).
84. Morimoto, K., Wolff, S., and Koizumi, A. Induction of sister-chromatid exchanges in human lymphocytes by microsomal activation of benzene metabolites. Mutat. Res. 119: 355-360 (1983).
85. Erexson, G. L., Wilmer, J. L., and Kligerman, A. D. Sister chromatid exchange induction in human lymphocytes exposed to benzene and its metabolites *in vitro*. Cancer Res. 45: 2471-2477 (1985).
86. Danford, N. D. Tests for chromosome aberrations and aneuploidy in the Chinese hamster fibroblast cell line CH1-L. In: Evaluation of Short-Term Tests for Carcinogens: Report of the International Program on Chemical Safety Collaborative Study on In Vitro Assays (J. Ashby, F. J. deSerres, M. Draper, M. Ishidate, Jr., B. H. Margolin, B. E. Matter, and M. D. Shelby, Eds.), Elsevier, Amsterdam, 1985, pp. 397-411.
87. Amacher, D. E., and Zelljadt, I. The morphological transformation of Syrian hamster embryo cells by chemicals reportedly nonmutagenic to *Salmonella typhimurium*. Carcinogenesis 4: 291-295 (1983).
88. Barrett, J. C., and Lamb, P. W. Tests with the Syrian hamster embryo cell transformation assay. In: Evaluation of Short-Term Tests for Carcinogens: Report of the International Program on Chemical Safety Collaborative Study on In Vitro Assays (J. Ashby, F. J. deSerres, M. Draper, M. Ishidate, Jr., B. H. Margolin, B. E. Matter, and M. D. Shelby, Eds.), Elsevier. Amsterdam, 1985, pp. 623-628.
89. Sanner, T., and Rivedal, E. Tests with the Syrian hamster embryo (SHE) cell transformation assay. In: Evaluation of Short-Term Tests for Carcinogens: Report of the International Program on Chemical Safety Collaborative Study on In Vitro Assays (J. Ashby, F. J. deSerres, M. Draper, M. Ishidate, Jr., B. H. Margolin, B. E. Matter, and M. D. Shelby, Eds.), Elsevier, Amsterdam, 1985, pp. 665-671.
90. McGregor, D., and Ashby, J. Summary report on the performance of the cell transformation assays. In: Evaluation of Short-Term Tests for Carcinogens: Report of the International Program on Chemical Safety Collaborative Study on In Vitro Assays (J. Ashby, F. J. deSerres, M. Draper, M. Ishidate, Jr., B. H. Margolin, B. E. Matter, and M. D. Shelby, Eds.), Elsevier, Amsterdam, 1985, pp. 103-115.
91. Díaz, M., Reiser, A., Braier, L., and Diez, J. Studies on benzene mutagenesis. I. The micronucleus test. Experientia 36: 297-299 (1980).
92. Tunek, A., Högstedt, B., and Olofsson, T. Mechanism of benzene toxicity. Effects of benzene and benzene metabolites on bone marrow cellularity, number of granulopoietic stem cells and frequency of micronuclei in mice. Chem.-Biol. Interact. 39: 129-138 (1982).
93. Hite, M., Pecharo, M., Smith, I., and Thorton, S. Effect of benzene in the micronucleus test. Mutat. Res. 77: 149-155 (1980).
94. Meyne, J., and Legator, M. S. Sex-related differences in cytogenetic effects of benzene in the bone marrow of Swiss mice. Environ. Mutagen. 2: 43-50 (1980).
95. Siou, G., Conan, L., and el Haitem, M. Evaluation of the clastogenic action of benzene by oral administration with 2 cytogenetic techniques in mouse and Chinese hamster. Mutat. Res. 90: 273-278 (1981).
96. Gad-El-Karim, M. M., Harper, B. L., and Legator, M. S. Modifications in the myeloclastogenic effect of benzene in mice with toluene, phenobarbitol, 3-methylcholanthrene, Aroclor 1254 and Skf-525A. Mutat. Res. 135: 225-243 (1984).
97. Choy, W. N., MacGregor, J. T., Shelby, M. D., and Maronpot, R. R. Induction of micronuclei by benzene in B6C3F$_1$ mice: retrospective analysis of peripheral blood smears from the NTP carcinogenesis bioassay. Mutat. Res. 143: 55-59 (1985).
98. Gad-El Karim, M. M., Sadagopa Ramanujam, V. M., and Legator, M. S. Correlation between the induction of micronuclei in bone marrow by benzene exposure and the excretion of metabolites in urine of CD-1 mice. Toxicol. Appl. Pharmacol. 85: 464-477 (1986).
99. Harper, B. L., and Legator, M. S. Pyridine prevents the clastogenicity of benzene but not of benzo(a)pyrene or cyclophosphamide. Mutat. Res. 179: 23-31 (1987).

100. Erexson, G. L., Wilmer, J. L., Steinhagen, W. H., and Kligerman, A. D. Induction of cytogenetic damage in rodents after short-term inhalation of benzene. Environ. Mutagen. 8: 29–40 (1986).
101. Tice, R. R., Costa, D. L., and Drew, R. T. Cytogenetic effects of inhaled benzene in murine bone marrow: induction of sister chromatid exchanges, chromosomal aberrations, and cellular proliferation inhibition in DBA/2 mice. Proc. Natl. Acad. Sci. (U.S.) 77: 2148–2152 (1980).
102. Tice, R., Fogt, T., and Costa, D. Cytogenetic effects of inhaled benzene in murine bone marrow. In: Genetoxic Effects of Airborn Agents (R. Tice, D. Costa, and K. Schaich, Eds.), Plenum Press, New York, 1982, pp. 257–275.
103. Rithidech, K., Au, W. W., Ramanujam, V. M., Whorton, E. B., Jr., and Legator, M. S. Induction of chromosome aberrations in lymphocytes of mice after subchronic exposure to benzene. Mutat. Res. 188: 135–140 (1987).
104. Dean, B. Chemical-induced chromosome damage. Lab. Anim. 3: 157–174 (1969).
105. Anderson, D., and Richardson, C. Issues relevant to the assessment of chemically induced chromosome damage *in vivo* and their relationship to chemical mutagenesis. Mutat. Res. 90: 261–272 (1981).
106. Styles, J. A., and Richardson, C. R. Cytogenetic effects of benzene: dosimetric studies on rats exposed to benzene vapour. Mutat. Res. 135: 203–209 (1984).
107. Kissling, M., and Speck, B. Chromosome aberrations in experimental benzene intoxication. Helv. Med. Acta. 36: 59–66 (1971).
108. Topham, J. Do induced sperm-head abnormalities in mice specifically identify mammalian mutagens rather than carcinogens? Mutat. Res. 74: 379–387 (1980).
109. Watanabe, G.-I., and Yoshida, S. The teratogenic effect of benzene in pregnant mice. Acta Med. Biol. (Niigata)17(1): 285–291 (1970).
110. Green, J., Leong, B., and Laskin, S. Inhaled benzene fetotoxicity in rats. Toxicol. Appl. Pharmacol. 46: 9–18 (1978).
111. Kuna, R., and Kapp, R. The embryotoxic/teratogenic potential of benzene vapor in rats. Toxicol. Appl. Pharmacol. 57: 1–7 (1981).
112. Hudák, A., and Ungváry, G. Embryotoxic effects of benzene and its methyl derivatives: toluene, xylene. Toxicology 11: 55–63 (1978).
113. Ungváry, G., and Tátrai, E. On the embryotoxic effects of benzene and its alkyl derivatives in mice, rats, and rabbits. Arch. Toxicol. (Suppl. 8): 425–430 (1985).
114. Coate, W. B., Hoberman, A. M., and Durloo, R. S. Inhalation teratology study of benzene in rats. Adv. Mod. Environ. Toxicol. 6: 187–198 (1984).
115. Ungváry, G. The possible contribution of industrial chemicals (organic solvents) to the incidence of congenital defects caused by teratogenic drugs and consumer goods: an experimental study. Prog. Clin. Biol. Res 163B: 295–300 (1985).
116. Keller, K. A., Snyder, C. A., Dempster, A. M., and Valle, C. D. Effects of benzene on fetal hematopoiesis. Teratology 31: 28A–29A (1985).
117. Keller, K. A., and Snyder, C. A. Mice exposed in utero to low concentrations of benzene exhibit enduring changes in their colony forming hematopoietic cells. Toxicology 42: 171–181 (1986).
118. Ward, C. O., Kuna, R. A., Snyder, N. K., Alsaker, R. D., Coate, W. B., and Craig, P. H. Subchronic inhalation toxicity of benzene in rats and mice. Am. J. Ind. Med. 7: 457–473 (1985).
119. American Petroleum Institute (API). Inhalation Teratology Study in Rats: Benzene. API Med. Res. Publ. No. 30-30224 (1982).
120. Schwetz, B. A review of the developmental toxicity of benzene. Advances in Modern Environmental Toxicology (M. Mehlman, Ed.), Princeton Scientific Publishers, Princeton, NJ, 1983, pp. 17–21.
121. Mehlman, M., Schreiner, C., and Mackerer, C. Current status of benzene teratology: a brief review. J. Environ. Pathol. Toxicol. 4: 123–131 (1981).
122. Davis, D. Reproductive risks of benzene: need for additional study. Toxicol. Ind. Health 2(4): 445–451 (1986).
123. Agency for Toxic Substances and Diseases Registry (ATSDR). Toxicological Profile for Benzene. ATSDR, U. S. Public Health Service, Atlanta, GA, 182 pp. 1988.
124. IARC. Monographs on the Evaluation of Carcinogenic Risks to Humans. Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs, Volumes 1 to 42. Supplement 7: Benzene 120–122. International Agency for Research on Cancer, Lyon, France, 1987.
125. Cronkite, E. P., Drew, R. T., Inoue, T., Hirabayashi, Y., and Bullis, J. E. Hematoxicity and carcinogenicity of inhaled benzene. Environ. Health Perspect. 82: 97–108 (1989).
126. Maltoni, C., Ciliberti, A., Cotti, G., Contri, B., and Belpoggi, F. Benzene, an experimental multipotential carcinogen: Results of the long-term bioassays performed at the Bologna Institute of Oncology. Environ. Health Perspect. 82: 109–124 (1989).
127. NCI. Bioassay of Phenol for Possible Carcinogenicity. Technical Report No. 203. National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, MD, 1980, p. 123.
128. Huff, J. E. Carcinogenesis results on seven amines, two phenols, and one diisocyanate used in plastics and synthetic elastomers. In: Industrial Hazards of Plastics and Synthetic Elastomers (J. Jarvisalo, P. Pfaffli, and H. Vainio, Eds.), Alan R. Liss, Inc., New York, 1984, pp. 347–363.
129. Salaman, M., and Glendenning, O. Tumour promotion in mouse skin by sclerosing agents. Br. J. Cancer 11: 434–444 (1957).
130. Boutwell, R., and Bosch, D. The tumor-promoting action of phenol and related compounds for mouse skin. Cancer Res. 19: 413–424 (1959).
131. Wynder, E., and Hoffman, D. A study of tobacco carcinogenesis. VIII. The role of the acidic fractions as promoters. Cancer 14: 1306–1315 (1969).
132. Van Duuren, B., Sivak, A., Langseth, L., Goldschmidt, B., and Segal, A. Initiators and promoters in tobacco carcinogenesis. Toward a less harmful cigarette. National Cancer Institute Monograph 28, U. S. Department of Health, Education, and Welfare, Public Health Service, National Cancer Institute, 1968, pp. 173–180.
133. Van Duuren, B., and Goldschmidt, B. Cocarcinogenic and tumor-promoting agents in tobacco carcinogenesis. J. Natl. Cancer Inst. 56: 1237–1242 (1976).
134. Van Duuren, B., Katz, C., and Goldschmidt, B. Brief communication: cocarcinogenic agents in tobacco carcinogenesis. J. Natl. Cancer Inst. 51: 703–705 (1973).
135. Lehman, A., Fitzhugh, O., Nelson, A., and Woodard, G. The pharmacological evaluation of antioxidants. Adv. Food Res. 3: 197–208 (1951).
136. Hecht, S., Thorne, R., Maronpot, R., and Hoffman, D. A study of tobacco carcinogenesis. XIII. Tumor-promoting subfractions of the weakly acidic fraction. J. Natl. Cancer Inst. 55: 1329–1336 (1975).
137. Hecht, S., Carmella, S., Mori, H., and Hoffman, D. A study of tobacco carcinogenesis. XX. Role of catechol as a major cocarcinogen in the weakly acidic fraction of smoke condensate. J. Natl. Cancer Inst. 66: 163–169 (1981).
138. Boyland, E., Busby, E., Dukes, C., Grover, P., and Manson, D. Further experiments on implantation of materials into the urinary bladder of mice. Br. J. Cancer 18: 575–581 (1964).
139. Hirose, M., Kurata, Y., Tsuda, H., Fukushima, S., and Ito, N. Catechol strongly enhances rat stomach carcinogenesis: a possible new environmental stomach carcinogen. Japan J. Cancer Res. (Gann) 78: 1144–1149 (1987).
140. Carlson, S. J., and Brewer N. R. Toxicity studies on hydroquinone. Proc. Soc. Exp. Biol. 84: 684–688 (1953).
141. Roe, F., and Salaman, M. Further studies on incomplete carcinogenesis: Triethylene melamine (T.E.M.), 1,2-benzanthracene and β-propiolactone as initiators of skin tumour formation in the mouse. Br. J. Cancer 9: 177–203 (1955).
142. Kari, F. Toxicology and Carcinogenesis Studies of Hydroquinone (CAS No. 123-31-9) in F344/N Rats and B6C3F$_1$ Mice (gavage studies). Technical Report No. 366. National Toxicology Program/National Institute of Environmental Health Sciences, Research Triangle Park, NC, 1988, pp. 296.
143. IARC. Catechol, Hydroquinone, and *para*-Quinone. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man. Some Fumigants, the Herbicides 2,4-D and 2,4,5-T, Chlorinated Dibenzodioxins and Miscellaneous Industrial Chemicals. International Agency for Research on Cancer, Lyon, France, 1977, pp. 155–175, 255–264.

144. Kishizawa, F. Carcinogenic action of *para*-benzoquinone on the lung of mice by the experimental inhalation (Report 1). Gann 45: 389-391 (1954).
145. Kishizawa, F. Carcinogenic action of benzoquinone on the lung of mice by the experimental inhalation (Report 2). Gann 46: 359-361 (1955).
146. Kishizawa, F. Carcinogenic action of *para*-benzoquinone on the lung of mice by the experimental inhalation (Report 3). Gann 47: 601-603 (1956).
147. Umeda, M. Production of rat sarcoma by injections of propylene glycol solution of *p*-quinone. Gann 48: 139-144 (1957).
148. Eastin, W. Toxicology and Carcinogenesis Studies of Xylenes (mixed) (60.2% *M*-Xylene, 13.6% *m*Xylene, 9.1% *o*-Xylene, and 17.0% Ethylbenzene) (CAS No. 1330-20-7) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies). Technical Report No. 327. National Toxicology Program/National Institute of Environmental Health Sciences, Research Triangle Park, NC, 1986, pp. 160.
149. Huff, J. E. Toxicology and Carcinogenesis Studies of Toluene (CAS No. 108-88-3) in F344/N Rats and B6C3F$_1$ Mice (Inhalation Studies). Technical Report No. 371. National Toxicology Program/National Institute of Environmental Health Sciences, Research Triangle Park, NC (1989).
150. Goldstein, B. Benzene Toxicity: Review of Recent Literature. Report prepared for the American Petroleum Institute (Feb. 3, 1983).
151. Infante, P. Leukemia among workers exposed to benzene. Tex. Rep. Biol. Med. 37: 153-161 (1978).
152. Infante, P. F., and White, M. C. Benzene: epidemiologic observations of leukemia by cell type and adverse health effects associated with low-level exposure. Environ. Health Perspect. 52: 75-82 (1983).
153. Grossenbacher, J., and Lob, M. Occupational exposure to benzene: follow-up study (Abst.). Schweiz. Med. Wochenschr. 112: 1858 (1982).
154. Santesson, C. Chronic poisoning with coal tar benzene; four deaths. Clinical and pathological-anatomical observations of several colleges and illustrating animal experiments (Ger.). Arch. Hyg. (Munchen) 31: 336-376 (1897).
155. Deutsche Forschungsgemeinschaft. Benzol am arbeitsplatz (Benzene in the workplace). Weinheim, Verlag Chemie Gmbh (1974).
156. Flury, F. II. Modern occupational intoxications. II(a). Modern occupational intoxications from the aspect of pharmacology and toxicology. Arch. Exp. Pathol. Pharmakol. 138: 65-82 (1928).
157. Snyder, R., and Kocsis, J. Current concepts of chronic benzene toxicity. Crit. Rev. Toxicol. 3: 265-288 (1975).
158. Sarto, F., Cominato, I., Pinton, A., Brovedani, P., Merier, E., Peruzzi, M., Bianchi, V., and Levis, A. A cytogenetic study on workers exposed to low concentrations of benzene. Cytogenetics 5: 827-832 (1984).
159. Delore, P., and Borgomano, C. Acute leukemia following benzene poisoning. On the toxic origin of certain acute leukaemias and their relation to serious anaemias. (Fr.) J. Med. Lyon 9: 227-233 (1928).
160. Goldstein, B. Hematoxicity in humans. J. Toxicol. Environ. Health 2: 69-105 (1977).
161. Vigliani, E. Leukemia associated with benzene exposure. Ann. N. Y. Acad. Sci. 271: 143-151 (1976).
162. Infante, P., Rinsky, R., Wagoner, J., and Young, R. Leukemia in benzene workers. Lancet ii: 76-78 (1977).
163. Rinsky, R. A., Smith, A. B., Hornung, R., Filloon, T. G., Young, R. J., Okun, A. H., and Landrigan, P. J. Benzene and leukemia. An epidemiologic risk assessment. New England J. Med. 316: 1044-1050 (1986).
164. Ott, M. G., Townsend, J. C., Fishbeck, W. A., and Langer, R. A. Mortality among individuals occupationally exposed to benzene. Arch. Environ. Health 33: 3-10 (1978).
165. Decouflé, P., Blattner, W. A., and Blair, A. Mortality among chemical workers exposed to benzene and other agents. Environ. Res. 30: 16-25 (1983).
166. Aksoy, M. Malignancies due to occupational exposure to benzene. Am. J. Ind. Med. 7: 395-402 (1985).
167. Infante, P. F., and White, M. C. Projections of leukemia risk associated with occupational exposure to benzene. Am. J. Ind. Med. 7: 403-413 (1985).
168. Aksoy, M. Hematotoxicity and carcinogenicity of benzene. Environ. Health Perspect. 82: 193-197 (1989).
169. Yin, S., Li, G.-L., Tain, F.-D., Fu, Z.-I., Jin, C., Chen, Y.-J., Luo, S.-J., Ye, P.-Z., Zhang, J.-Z., Wang, G.-C., Zhang, X.-C., Wu, H.-N., and Zhong, Q.-C. A retrospective cohort study of leukemia and other cancers in benzene workers. Environ. Health Perspect. 82: 207-213 (1989).
170. Austin, H., Delzell, E., and Cole, P. Benzene and leukemia. A review of the literature and a risk assessment. Am. J. Epidemiol. 127: 419-439 (1988).
171. Goldstein, B. Risk assessment and risk management of benzene by the Environmental Protection Agency. In: Risk Quantitation and Regulatory Policy, Banbury Report 19 (D. G. Hoel, R. A. Merrill, and F. P. Perera, Eds.), Cold Spring Harbor Laboratory, 1985, pp. 293-304.
172. Maronpot, R. R., and Boorman, G. A. Interpretation of rodent hepatocellular proliferative alterations and hepatocellular tumors in chemical safety assessment. Toxicol. Pathol. 10: 71-80 (1982).
173. Boorman, G. A., Montgomery, C. A., Jr., Eustis, S. L., Wolfe, M. J., McConnell, E. E., and Hardisty, J. F. Quality assurance in pathology for rodent carcinogenicity studies. In: Handbook of Carcinogen Testing (H. Milman and E. Weisburger, Eds.), Noyes Publications, Park Ridge, NJ, 1985, pp. 345-357.
174. McConnell, E. E., Solleveld, H. A., Swenberg, J. A., and Boorman, G. A. Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. J. Natl. Cancer Inst. 76: 283-289 (1986).
175. Kaplan, E. L., and Meier, P. Nonparametric estimation of incomplete observations. J. Am. Stat. Assoc. 53: 457-481 (1958).
176. Cox, D. R. Regression models and life tables. J. R. Stat. Soc. B34: 187-220 (1972).
177. Tarone, R. E. Tests for trend in life table analysis. Biometrika 62: 679-682 (1975).
178. Armitage, P. Statistical Methods in Medical Research. John Wiley & Sons, New York, 1971, pp. 362-365.
179. Gart, J. J., Chu, K. C., and Tarone, R. E. Statistical issues in interpretation of chronic bioassay tests for carcinogenicity. J. Natl. Cancer Inst. 62: 957-974 (1979).
180. Haseman, J. K. Statistical issues in the design, analysis and interpretation of animal carcinogenicity studies. Environ. Health Perspect. 58: 385-392 (1984).
181. Winer, B. Statistical Principles in Experimental Design. McGraw-Hill, New York, 1971, pp. 518-539.
182. Dunnett, C. A multiple comparison procedure for comparing several treatments with a control. J. Am. Stat. Assoc. 50: 1096-1121 (1955).
183. Dunnett, C. New tables for multiple comparisons with a control. Biometrics 20: 482-491 (1964).
184. Haseman, J. K., Huff, J. E., and Boorman, G. A. Use of historical control data in carcinogenicity studies in rodents. Toxicol. Pathol. 12: 126-135 (1984).
185. Haseman, J. K., Huff, J. E., Rao, G. N., Arnold, J., Boorman, G. A., and McConnell, E. E. Neoplasms observed in untreated and corn oil gavage control groups of F344/N rats and (C57BL/6N x C3H/HeN)F$_1$ (B6C3F$_1$) mice. J. Natl. Cancer Inst. 75: 975-984 (1985).
186. Pliss, G. Tumours of the auditory sebaceous glands. In: Pathology of Tumours in Laboratory Animals, Vol. I. Tumours of the Rat (V. Turusov, Ed.), IARC Sci. Pub. 6, International Agency for Research on Cancer, Lyon, France, 1973, pp. 23-30.
187. Pohl, R. J., and Fouts, J. R. Cytochrome P-450-dependent xenobiotic metabolizing activity in Zymbal's gland, a specialized sebaceous gland of rodents. Cancer Res. 43: 3660-3662 (1983).
188. Maltoni, C., and Scarnato, C. First experimental demonstration of the carcinogenic effects of benzene; long-term bioassays on Sprague-Dawley rats by oral administration. Med. Lav. 70: 352-357 (1979).
189. Maltoni, C., Conti, B., Cotti, G., and Belpoggi, F. Experimental studies on benzene carcinogenicity at the Bologna Institute of Oncology: Current results and ongoing research. Am. J. Ind. Med. 7: 415-446 (1985).
190. Parke, D. V., and Williams, R. T. The metabolism of benzene containing [^{14}C$_1$]benzene. Biochem. J. 54: 231-238 (1953).
191. Snyder, R. Relation of benzene metabolism to benzene toxicity. In: Symposium on Toxicology of Benzene and Alkylbenzenes

(D. Braun, Ed.), Pittsburgh, PA, 1974, p. 44.
192. Dorland Illustrated Medical Dictionary, 25th ed. W. B. Saunders Co., Philadelphia, PA, 1974.
193. Tucker, M. Tumours of the Harderian gland. In: Pathology of Tumours in Laboratory Animals, Vol. II. Tumours of the Mouse (V. Turusov, Ed.), IARC Sci. Pub. 23, International Agency for Research on Cancer, Lyon, France, 1979, pp. 135–145.
194. Gibson, D., Cardini, G., Maseles, F., and Kallio, R. Oxidative degradation of aromatic hydrocarbons by microorganisms. IV. Incorporation of oxygen-18 into benzene by *Pseudomonas putida*. Biochemistry 9: 1631–1635 (1970).
195. Goldstein, B. D., Snyder, C. A., Laskin, S., Bromberg, I., Albert, R. E., and Nelson, N. Myelogenous leukemia in rodents inhaling benzene. Toxicol. Lett. 13: 169–173 (1982).
196. Neuberger, A., and Smith, R. Richard Tecwyn Williams: the man, his work, his impact. Drug Metab. Rev. 14: 559–607 (1983).
197. Andrews, L., Sasame, H., and Gillette, J. ^{3}H-Benzene metabolism in rabbit bone marrow. Life Sci. 25: 567–572 (1979).
198. Irons, R. D., Dent, J. G., Baker, T. S., and Rickert, D. E. Benzene is metabolized and covalently bound in bone marrow in situ. Chem.-Biol. Interact. 30: 241–245 (1980).
199. Rickert, D. E., Baker, T. S., Bus, J. S., Barrow, C. S., Irons, R. D. Benzene disposition in the rat after exposure by inhalation. Toxicol. Appl. Pharmacol. 49: 417–423 (1979).
200. Greenlee, W. F., Gross, E. A., and Irons, R. D. Relationship between toxicity and disposition of ^{14}C-labeled benzene metabolites in the rat. Chem.-Biol. Interact. 33: 285–299 (1981).
201. Proctor, B. L., Gaulden, M. E., and Dowd, M. A. Reactivity and fate of benzene and formaldehyde in culture medium with and without fetal calf serum; relevance to in vitro mutagenicity testing. Mutat. Res. 160: 259–266 (1986).
202. Gocke, E., King, M.-T., Eckardt, K., and Wild, D. Mutagenicity of cosmetics ingredients licensed by the European communities. Mutat. Res. 90: 91–109 (1981).
203. Gad-el-Karim, M. M., Ramanujam, V. M., Ahmed, A. E., and Legator, M. S. Benzene myeloclastogenicity: a function of its metabolism. Am. J. Ind. Med. 7: 475–484 (1985).
204. Epler, J., Larimer, F., Rao, T., Nix, C., and Ho, T. Energy-related pollutants in the environment: use of short-term tests for mutagenicity in the isolation and identification of biohazards. Environ. Health Perspect. 27: 11–20 (1978).
205. Stich, H., Rosin, M., Wu., C., and Powrie, W. The action of transition metals on the genotoxicity of simple phenols, phenolic acids and cinnamic acids. Cancer Lett. 14: 251–260 (1981).
206. Garberg, P., Akerblom, E.-L., and Bolcsfoldi, G. Evaluation of a genotoxicity test measuring DNA-strand breaks in mouse lymphoma cells by alkaline unwinding and hydroxylapatite elution. Mutat. Res. 203: 155–176 (1988).
207. Crebelli, R., Conti, G., and Carere, A. On the mechanism of mitotic segregation induction in *Aspergillus nidulans* by benzene hydroxy metabolites. Mutagenesis 2: 235–238 (1987).
208. Selkirk, J. K., Croy, R. G., Whitlock, J. P., Jr., and Gelboin, H. V. In vitro metabolism of benzo(a)pyrene by human-liver microsomes and lymphocytes. Cancer Res. 35: 3651–3655 (1975).
209. Nebert, D., and Jensen, N. The *Ah* locus: genetic regulation of the metabolism of carcinogens, drugs, and other environmental chemicals by cytochrome P-450-mediated monooxygenases. Crit. Rev. Biochem. 6: 401–437 (1979).
210. Tunek, A., Platt, K., Przybylski, M., and Oesch, F. Multi-step metabolic activation of benzene. Effect of superoxide dismutase on covalent binding to microsomal macromolecules, and identification of glutathione conjugates using high pressure liquid chromatography and field desorption mass spectrometry. Chem.-Biol. Interact. 33: 1–17 (1980).
211. Tunek, A., Platt, K., Bentley, P., and Oesch, F. Microsomal metabolism of benzene to species irreversibly binding to microsomal protein and effects of modifications of this metabolism. Mol. Pharmacol. 14: 920–929 (1978).
212. Dean, B. Genetic toxicology of benzene, toluene, xylenes and phenols. Mutat. Res. 47: 75–97 (1978).
213. Cronkite, E. P., Bullis, J. E., Inoue, T., and Drew, R. T. Benzene inhalation produces leukemia in mice. Toxicol. Appl. Pharmacol. 75: 358–361 (1984).
214. Cronkite, E. P., Drew, R. T., Inoue, T., Bullis, J. E. Benzene hematotoxicity and leukemogenesis. Am. J. Ind. Med. 7: 447–456 (1985).
215. Post, G. B., Snyder, R., and Kalf, G. F. Inhibition of RNA synthesis and interleukin-2 production in lymphocytes in vitro by benzene and its metabolites, hydroquinone and *p*-benzoquinone. Toxicol. Lett. 29: 161–167 (1985).
216. Pellack-Walker, P., Walker, J. K., Evans, H. H., and Blumer, J. L. Relationship between the oxidation potential of benzene metabolites and their inhibitory effect on DNA synthesis in L5178YS cells. Mol. Pharmacol. 28: 560–566 (1985).
217. Snyder, R., Jowa, L., Witz, G., Kalf, G., and Rushmore, T. Formation of reactive metabolites from benzene. Arch. Toxicol. 60: 61–64 (1987).
218. Yunis, J. J. The chromosomal basis of human neoplasia. Science 221: 227–236 (1983).
219. Yunis, J. J., Soreng, A. L., and Bowe, A. E. Fragile sites are targets of diverse mutagens and carcinogens. Oncogene 1: 59–69 (1987).